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6968 C*

Comparison of Inorganic Phosphate Contents of Serum, Fluoride Plasma and Native Plasma.†

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In the course of work on the clearances of various urinary constituents (White and Monaghan[1]) we noted a difference between the inorganic phosphate values of serum and of fluoride plasma, where 0.25 to 0.3% NaF was used as anticoagulant. A few weeks later Gaebler[2] reported that 4.2% NaF inhibited or abolished the color production with the Benedict-Theis[3] or the Fiske-Subarrow[4] method and that this interference could be avoided by adding $AlCl_3$. Gaebler gives no data on the extent to which 1% NaF, the concentration which he used as anticoagulant, influences color production in the phosphate methods. Since, however, the lower phosphate content of fluoride plasma as compared with serum corresponded roughly with the extent to which the total nitrogen of the plasma was less than that of serum, it might be inferred that 1% NaF interferes little and that the lower phosphate content of the plasma is due in the main to its dilution by the osmotic effect of the fluoride.

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* P represents a preliminary, C a complete manuscript.

† This work was aided by a grant made by the Rockefeller Foundation to Washington University for research in science.

[1] White, H. L., and Monaghan, B., *Am. J. Physiol.*, 1933, **104**, 412.

[2] Gaebler, O. H., *J. Biol. Chem.*, 1932, **99**, 99.

[3] Benedict, S. R., and Theis, R. C., *J. Biol. Chem.*, 1924, **61**, 63.

[4] Fiske, C. H., and Subarrow, Y., *J. Biol. Chem.*, 1925, **66**, 375.

His results may have been somewhat complicated by the facts that the serum lacked fibrinogen nitrogen and that 1% NaF caused some hemolysis. Gaebler also reported that in 7 cases out of 8 serum separated after the blood had stood 30 minutes at about 25° showed a higher inorganic phosphate content than did native plasma separated immediately, while oxalate plasma always showed a lower phosphate content than native plasma. He suggests that serum is less suitable than native plasma for inorganic phosphate determinations.

We have (1) investigated the effect upon the color production of the addition of 0.3, 0.6, 0.9, and 1% NaF to known phosphate solutions and of 0.3% NaF to serum, (2) compared the inorganic P (Benedict-Theis method) and total N (macro Kjeldahl) contents of 0.3% fluoride plasma and of native plasma, both separated immediately, and in another set of experiments added hematocrit readings, substituting heparin or hirudin for native plasma, and (3) compared the inorganic P contents of serum and of native plasma, both separated immediately.

In the first case 5 cc. of phosphate standard containing 0.05 mg. P was compared with the same standard plus 3, 6, 9, and 10 mg. NaF. With the standard set at 20, the standard plus 3 mg. NaF read in duplicate 20.0 and 20.1, standard plus 6 mg. NaF 20.0 and 19.8, standard plus 9 mg. NaF 20.3 and 20.3, and standard plus 10 m. NaF 20.4 and 20.7. A dog serum gave in duplicate 4.14 and 4.14 mg. P per 100 cc. while with 0.3% added NaF it gave 4.06 and 4.15 mg. P per 100 cc.; a beef serum (in the refrigerator several days and showing considerable hemolysis had 7.82 and 7.70 mg. P per 100 cc. while with 0.3% NaF added it had 7.70 and 7.70 mg. per 100 cc. It is evident that the inhibiting effect of NaF is just beginning to manifest itself at a concentration of 0.9 or 1%.

To carry out the second and third comparisons referred to in the second paragraph we anesthetized dogs with sodium barbital and put a paraffined cannula into either a femoral or a carotid artery. At each experiment 3 blood samples were drawn within a few seconds. After washing out dead space blood one sample was received into a paraffined tube, one into a plain tube and one (15 cc.) into a tube containing 45 mg. NaF. All 3 were in the centrifuge in less than 2 minutes after being drawn and were centrifuged at 3000 r.p.m. for 5 minutes. With reasonable care in paraffining no difficulty was experienced in getting native plasma entirely free from coagulation. No hemolysis occurred in any samples of serum, fluoride plasma or native plasma. Phosphate determinations in duplicate were carried out by the Benedict-Theis method on all 3 samples immediately after centrifugation; in the last 7 experiments total N determinations were also carried out on native and fluoride plasma, 2 cc. being used for a macro Kjeldahl. Not all of the N determinations reported in Table I were made in duplicate; the N figures given are the averages of duplicates when these were run.

TABLE I
Mg. per 100 cc. average of duplicate determinations.

Exp. No.	Serum P	Native Plasma P	Fluoride Plasma P	Native Plasma N	Fluoride Plasma N	$\frac{\text{Native P}}{\text{Fluoride P}}$	$\frac{\text{Native N}}{\text{Fluoride N}}$
1	5.4	5.6	3.7			1.51	
2	5.5	5.7	4.7			1.21	
3	3.8	3.7	3.3			1.12	
4	3.9	3.9	lost				
5	5.6	5.6	5.1			1.10	
6	6.0	5.8	5.4			1.07	
7	3.9	3.8	3.5	905	820	1.09	1.10
8	2.5	2.5	2.2	995	897	1.12	1.11
9	4.6	4.5	4.1	998	903	1.10	1.11
10	3.2	3.3	2.9	965	885	1.13	1.09
11	3.4	3.3	3.1	928	883	1.06	1.05
12	4.7	4.6	4.2	938	874	1.10	1.08
13	5.0	5.0	4.4	945	823	1.14	1.15

It is seen in Table 1 that the phosphate content of 0.3% fluoride plasma is always lower than that of native plasma. In the last 7 experiments the phosphate differences correspond rather closely to the total nitrogen differences. Since we have shown that 0.3% NaF does not affect color production the most probable interpretation is that the lower phosphate value for fluoride plasma is due in most cases to dilution by the osmotic effect of the added fluoride. In 2 experiments, however, (1 and 2) and particularly in experiment 1, the P content of the fluoride plasma was so far below that of native plasma that it seemed improbable of explanation on the sole basis of osmotic dilution. It appeared that in the experiments of Table I the lower P content of fluoride plasma could usually but not always be accounted for by osmotic dilution.

In the second series of 6 experiments hematocrit readings were added to the total N and inorganic P determinations. Since paraffined tubes could not be used for hematocrit readings, heparin or hirudin plasma was substituted for native plasma. In the first experiment this was compared with 0.3% NaF plasma, in the second, third, and fourth with both 0.3% NaF plasma and with plasma from blood to which had been added 0.418% NaCl (isotonic with 0.3% NaF) and in the fifth and sixth with both 0.3% NaF plasma and plasma from blood to which had been added 0.876% potassium oxalate (isotonic with 0.3% NaF). The same amount of hirudin (0.2 mg. per cc.) or of heparin (0.2 mg. per cc.) was added to all the samples, including the fluoride, chloride and oxalate bloods. Hematocrit readings, total N by macro Kjeldahl and inorganic P determinations were done in duplicate on all samples; the figures are the averages of these duplicates.

TABLE II

Ex. No.	Added	% plasma	Total N mg. per 100 cc.	Inorganic P mg. per 100 cc.	% $\frac{\text{plasma}^2}{\text{plasma}^1}$	$\frac{N^1}{N^2}$	$\frac{P^1}{P^2}$	% $\frac{\text{plasma}^3}{\text{plasma}^1}$	$\frac{N^1}{N^3}$	$\frac{P^1}{P^3}$
1	hirudin (1)	63.7	992	4.7	1.09	1.06	1.27			
	fluoride (2)	69.4	937	3.7						
2	heparin (1)	63.2	938	7.6	1.11	1.08	1.21	1.10	1.06	1.10
	fluoride (2)	70.0	866	6.3						
	chloride (3)	69.4	883	6.9						
3	heparin (1)	63.7	913	7.0	1.11	1.03	1.13	1.10	1.12	1.06
	fluoride (2)	70.4	884	6.2						
	chloride (3)	69.8	813	6.6						
4	hirudin (1)	65.0	1056	5.8	1.09	1.09	1.11	1.09	1.11	1.09
	fluoride (2)	70.5	965	5.2						
	chloride (3)	70.9	950	5.3						
5	hirudin (1)	62.4	1039	6.7	1.08	1.10	1.13	1.12	1.13	1.24
	fluoride (2)	67.4	946	5.9						
	oxalate (3)	69.7	919	5.4						
6	hirudin (1)	64.6	1041	7.0	1.07	1.12	1.09	1.12	1.13	1.25
	fluoride (2)	69.3	928	6.4						
	oxalate (3)	72.3	922	5.6						

The results given in Table II show that the degree of dilution of the plasma as indicated by the total N figures usually corresponds with that shown by the hematocrit readings. In 7 cases out of 11 the drop in the P content of plasma from blood to which a salt has been added corresponds fairly closely to the degree of dilution of the plasma. In 4 cases the drop in P content is definitely greater than can be accounted for by dilution; 2 of these cases are with fluoride plasma and 2 with oxalate plasma. Neither fluoride nor oxalate in the concentrations used influences the color production of the P method. The findings of Table II constitute an extension and confirmation of those in Table I, showing that the drop in P content of plasma when an anticoagulant salt is used is usually but not always accounted for in the main by the dilution effect. In the cases where the drop in plasma P is greater than can be accounted for by dilution the absolute amount of inorganic P in the plasma has apparently decreased; the mechanism is not clear. In the 3 cases where a salt not an anticoagulant (NaCl) was used the drop in plasma P corresponds rather well with the degree of dilution; whether this would hold consistently in a large series we cannot say.

We have found, as did Gaebler for heparin, that both heparin and hirudin may transmit some material to a trichloracetic filtrate which causes difficulty in matching colors. This difficulty appears to a varying extent in a series of plasmas compared with a given lot of heparin or hirudin but is in no case so great as to introduce any large error. We obtained no hemolysis with 0.3% NaF, very slight with 0.876% potassium oxalate and definite hemolysis with 0.418% NaCl.

Table I also shows, contrary to Gaebler's observations, that there is no significant difference between the inorganic P content of serum and of native plasma. This may be due to our having centrifuged both blood samples immediately, while Gaebler let his serum bloods stand for 30 minutes at about 25° before centrifugation.

Summary. A concentration of 0.3% NaF does not influence the color production with the Benedict-Theis method for inorganic phosphates; an inhibiting effect is beginning to manifest itself with 1% NaF.

Plasma obtained by adding 0.3% NaF to blood shows a lower content of inorganic P than does native plasma or hirudin plasma. This difference is usually but not always accounted for by the osmotic dilution due to the added salt, the extent of dilution being measured by total N and hematocrit findings. In the cases where the drop in plasma P is greater than can be accounted for by dilution, the absolute amount of inorganic P in the plasma is apparently decreased.

The inorganic P content of serum is the same as that of native plasma.

6969 C

Proportion of Cystine Yielded by Hemoglobins of the Horse, Dog, and Sheep.*

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The presence of cystine among the products of hydrolysis of horse hemoglobin has escaped the notice of most investigators although Abderhalden,[1] about 30 years ago, reported the isolation of 0.3% from this protein. We have recently applied the cysteine cuprous mercaptide method[2] to the analysis of horse hemoglobin and have found 0.41% of cystine to be yielded by a highly purified preparation; it therefore seemed of interest to investigate the hemoglobins of other species, particularly in view of the fact that differences in sulfur content of various hemoglobins have long been a matter of record.

Hemoglobin was prepared from the blood of the sheep and the dog, and was recrystallized twice by the method of Hoppe-Seyler. Each preparation was dissolved in water and the solution was poured into a large volume of boiling water containing a trace of sodium chloride. The coagulated proteins were separately washed by repeated centrifugation with water until free from electrolytes, and were then dried in a current of warm air and ground to a fine powder.

The yield of cystine after hydrolysis in the presence of tin was determined by the cuprous mercaptide method,[2] iron was determined by the method of Kennedy[3] with the modifications of McFarlane,[4]‡ sulfur was determined by the method of Denis[5] after a preliminary oxidation of the protein with hot concentrated nitric acid, and nitrogen was determined by the Kjeldahl method with mercury as catalyst. The results of these analyses are shown in the table where they are calculated on an ash- and moisture-free basis.

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* The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington.

† Porter Fellow of the American Physiological Society.

[1] Abderhalden, E., *Z. physiol. Chem.*, 1903, **37**, 484.

[2] Vickery, H. B., and White, A., *J. Biol. Chem.*, 1933, **99**, 701.

[3] Kennedy, R. P., *J. Biol. Chem.*, 1927, **74**, 385.

[4] McFarlane, W. D., *Biochem. J.*, 1932, **26**, 1034.

‡ We are indebted to Dr. R. O. Brooke for the iron analyses.

[5] Denis, W., *J. Biol. Chem.*, 1910-11, **8**, 401.

TABLE I
Analysis of Hemoglobin.

	Cystine %	N %	S %	Fe %	Atomic ratio S: Fe
Horse	0.41	16.70	0.39	0.33	2.05
Sheep	0.60	16.83	0.73	0.32	4.00
	0.61				
Dog	1.17	16.48	0.57	0.33	3.02
	1.24				
	1.09				
	1.15				
Average	1.16				

The differences in the yields of cystine from these 3 hemoglobins are particularly striking and suggest that the yields of other amino acids from hemoglobins of different origin may likewise vary widely.

6970 C

Experimental Fetal Nephritis.

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The problem of uranium poisoning in the pregnant animal has been investigated by MacNider, Helms, and Helms,[1] who found that during gestation susceptibility to this poison is especially marked, and that the changes in the blood, urine, liver, and kidneys, as well as the clinical course, resemble certain major toxemias of pregnancy in man.

Our interest in this problem has been to determine whether the fetus likewise suffers from the intoxication. The series studied at Beirut comprised 4 dogs, 10 rabbits, one normal pregnant dog and one normal pregnant rabbit for control. More recently observations have been made on 2 dogs, 6 rabbits, and one cat. The results with the latter group are essentially the same as those in the first group, the data for which are outlined in Tables I and II. Uranium acetate was used in our experiment. MacNider and his associates employed uranium nitrate.

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[1] MacNider, W. deB., Helms, S. T., and Helms, S. C., *Bull. Johns Hopkins Hosp.*, 1927, **40**, 145.

Regarding the effect on the pregnant animal, our results confirm those of MacNider, Helms, and Helms, who employed injections of 4 mg. of uranium nitrate per kg. We used smaller doses in most of our experiments in an attempt to obtain living offspring and found that 1 mg. per kg. in the case of dogs was sufficient to produce a very marked intoxication.

TABLE I

Animal	Wt. Kg.	Uranium Acetate Amount Mg.	Injected Date	Delivery	Death
Dog 1	12	36	Oct. 10	Oct. 13, 4 puppies living, 1 dead.	Oct. 21, due to hemopericardium following heart puncture.
" 2	5.2	20.8	" 10	Oct. 17, dog moribund. Hysterectomy; 2 living puppies, 1 dead.	Oct. 18.
" 3	7.1	14.2	Nov. 14		Nov. 18, 4 fetuses near term; 1 macerated fetus.
" 4	11.6	11.6	" 2	Dec. 2, dog delivered a separated placenta; next morning 1 dead puppy found in cage.	Autopsied Jan. 18.
Rabbit 1	2.15	10	Oct. 13	Oct. 17-18, 3 dead young.	Oct. 21.
" 3	2	10	" 21	Oct. 22, premature separation of placenta, 1 dead fetus near term.	Oct. 27.
" 5	1.83	10	Oct. 31		Nov. 4, 5 fetuses removed.
" 8	2.36	5	Nov. 29	Dec. 5, 4 dead and 1 living young, appeared somewhat premature.	Dec. 6.

A brief statement of the microscopic findings is given in Table II. The changes in the glomeruli were found to be somewhat variable, both in the mother and young, but greater uniformity was noted in the case of the tubules. Such variations as occurred could be explained on the basis of such factors as dosage, duration of the intoxication, and the opportunity for reparative processes. Marked dilatation of the convoluted tubules (Fig. 1), suggestive of cystic kidney, was a frequent occurrence in the new born, especially in the puppies. This was apparently due to obliteration of the collecting

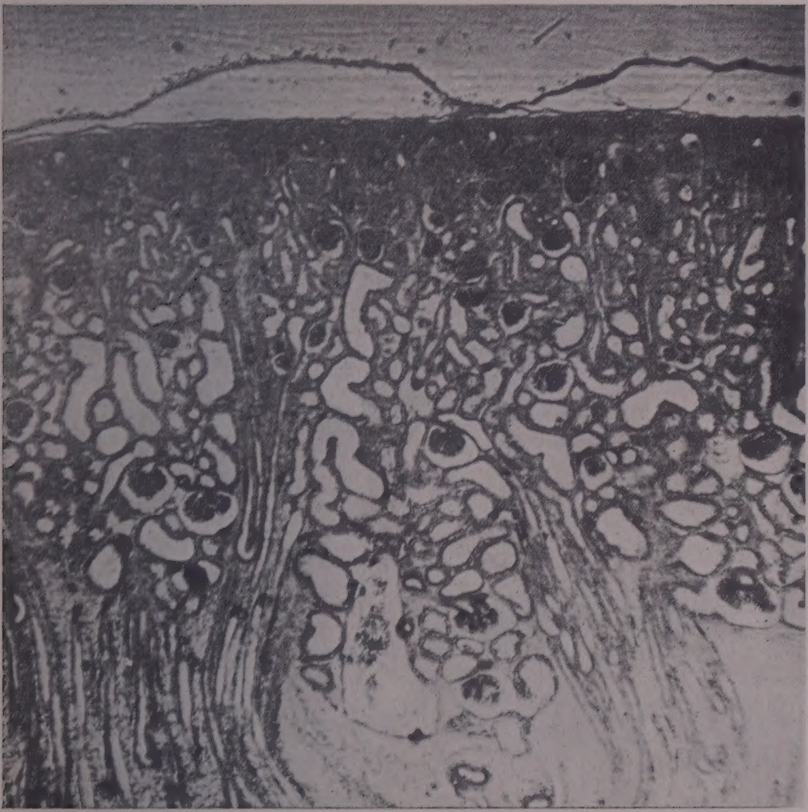

FIG. 1.
Dilatation of tubules and some dilatation of capsular spaces. Puppy 2 a. ×57. H and E stain.

tubules, at least in part, from compression by proliferating connective tissue (Fig. 2).

At birth the N.P.N. in the blood of the young was similar to that of the mother. Thus in Dog 2, the N.P.N. was found to be 368 mg. and that of the living puppies, 369 mg. Protein was present in the bladder urine of all newborn nephritic animals, but not in the controls.

In Dog 1 several hours before delivery the N.P.N was 77.6 mg. The litter was born during the night. On the following morning the N.P.N. in the blood of the puppies was 79.2 mg. and that of the mother, 173 mg. Three days later the mother's N.P.N was 216 mg., followed by a decrease on the seventh day to 182.5 mg.

TABLE II

Number	Glomeruli	Tubules	Blood Vessels	Interstitial Tissue
Dog 1	Slight exudate into capsular space.	Cloudy swelling and degeneration of convoluted tubules; granular casts in straight tubules; collapse of some.	Negative	Negative
Puppy 1a (dead at birth)	Tufts negative; Bowman's capsule distended with exudate.	Convoluted-cloudy swelling; some show degeneration, dilatation, hemorrhage into some.	Engorgement	Edema
Puppy 1b (living—autopsied 1st day)	Tufts negative; slight distension of Bowman's capsule.	Convoluted-cloudy swelling; some show degeneration, slight dilatation and few casts.	Negative	Increased cellularity, collapsed tubules in medulla.
Puppy 1c (found dead on 4th day and puppy 1d autopsied on 8th day).	Tufts negative; distension of Bowman's capsule with exudate.	Convoluted-cloudy swelling; some show degeneration, dilatation of some tubules.	"	Increased cellularity.
Dog 2	Tufts-partial destruction; capsules distended with exudate.	Convoluted-advanced cloudy swelling and degeneration, granular and hyaline casts.	Connective tissue around vessels increased.	Proliferation of fibroblasts in midzone.
	Patches of infarcts from cortex to medulla with connective tissue proliferation.			
Puppy 2a (living at birth).	Tufts negative; capsule-marked dilatation.	Convoluted-some cloudy swelling-marked dilatation of lumen.	Proliferation of fibroblasts around blood vessels especially in midzone.	Proliferation of fibroblasts in medulla around collecting tubules and ducts of Bellini.
Dog 3	Tufts-Partial destruction; capsule-exudate.	Convoluted-cloudy swelling, some show degeneration; casts in straight tubules.	Negative	Increase of connective tissue.
Puppy 3a	Tufts-congestion; capsule-exudate.	Convoluted-cloudy swelling and dilatation.	Engorgement	Congestion of medulla.
Dog 4	Tufts-increased cellularity; Bowman's capsule adhesions, partial obliteration, some have a little exudate.	Convoluted-beginning of process of regeneration in degenerated ones; absence of casts.	Increase of connective tissue around blood vessels.	Increase of collagen fibres around tubules in medulla.
			As seen by Mallory's aniline blue stain.	

Puppy 4a	Negative	Convoluted-cloudy swelling; some show degeneration, exudate and dilatation.	Negative	Proliferation of connective tissue in midzone (cortico-medullary zone).
Puppy (newborn normal)	Normal	Normal	Normal	Normal
Rabbit 1	Tufts-some destruction; capsule, some exudate.	Convoluted-extensive cloudy swelling and degeneration; collecting-granular casts.	Engorgement	Some leucocytic infiltration.
Young a and b	Tufts-some destruction; some show proliferation of epithelium and exudation.	Convoluted-cloudy swelling, dilatation of some; straight-collapse of some.	Negative	Proliferation of connective tissue in midzone.
Rabbit 3		Same as Rabbit 1		
Young a	Tufts-practically negative; capsules-some show exudate and some of these proliferation of the epithelium.	Convoluted-cloudy swelling and degeneration; beginning dilatation.	Congestion of medulla.	Proliferation of loose connective tissue in medulla
Rabbit 5	Slight involvement	Convoluted-advanced degeneration, collecting-abundant casts.	Some closed; some thickened.	Increase of connective tissue.
Young 5a	Tufts-negative; capsules-some show dilatation.	Convoluted-patches of hyaline degeneration and cloudy swelling, dilatation.	Negative	Negative
Rabbit 8	Partial to complete damage of glomeruli.	Convoluted-advanced degeneration; collecting-abundant casts.	,,	,,
Young 8a (born dead).	Incomplete development.	Convoluted-slight cloudy swelling; beginning dilatation.	,,	,,
Young 8b (born alive).	Tufts-practically normal; capsules-slight exudate into some.	Convoluted-marked swelling, more than 8a; dilatation more marked.	,,	,,
Rabbit (newborn normal)	Normal	Normal	Normal	Normal

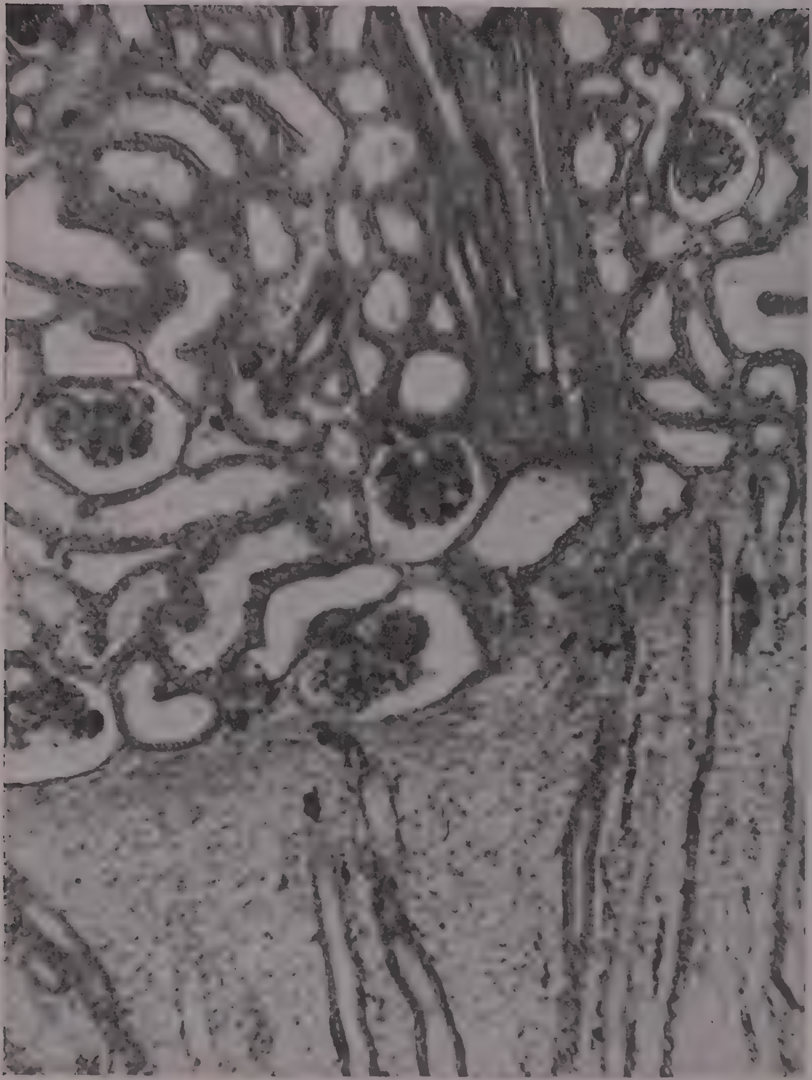

FIG. 2.

Dilatation of tubules, fibroblastic reaction of the midzonal region, together with compression of some of the tubules. Granular debris in convoluted tubules. Puppy 2 a. ×130. H and E stain.

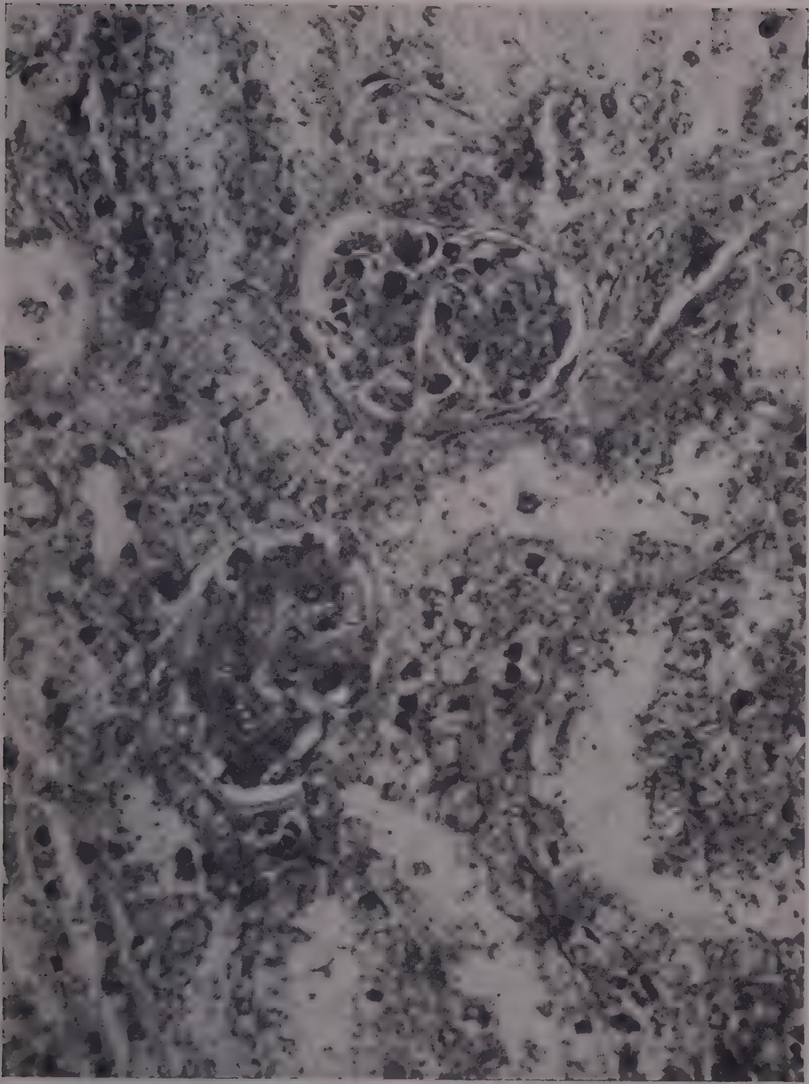

FIG. 3.

Relatively little change of the glomerular tuft, but marked changes in convoluted tubules; swollen granular cytoplasm and in some areas, loss of nuclei. Granular debris and desquamated epithelial cells in tubules. Puppy 1 d. × 480. H and E stain.

At this time the surviving puppy showed an N.P.N. of only 102 mg, suggesting a somewhat more rapid return to normal function.

6971 C

Effect of Antuitrin "S"* on Weights of the Pituitary, Adrenal and Thyroid.

GEORGE M. LEIBY. (Introduced by John C. Burch.)

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Histological and physiological variations of the pituitary as a result of urinary hebin have been observed by numerous workers. We wish to present evidence of morphological changes, indicated by organ weights, of the pituitary, adrenal and thyroid glands.

Castrate rats were used in order to eliminate the excessive ovarian secretions that result from injections of urinary hebin. A commercial preparation of urinary hebin (Antuitrin "S") was used.

Forty-two female adult albino rats were treated in 2 groups: Eight rats received 50 rat units of Antuitrin "S" on the twenty-fifth day after castration and daily for the ensuing 9 days. Twenty-seven rats received 25 rat units of Antuitrin "S" daily on the 22nd day after castration and for the following 5 days. At the end of the period of injection the glands were removed and weighed on a torsion balance.

Forty-six adult albino female rats were castrated in 3 groups and sacrificed as non-injected castrate controls as follows: 14 rats, 28

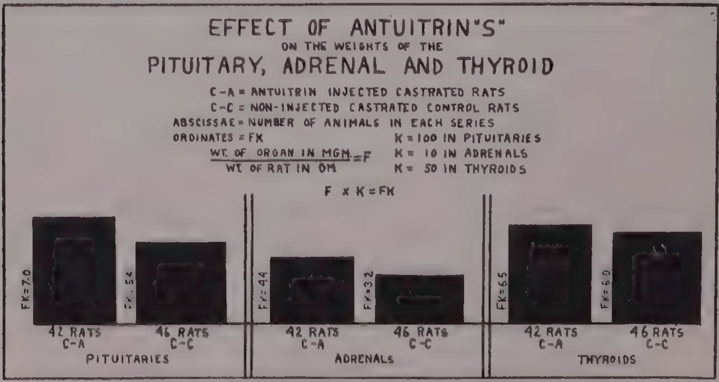

FIG. 1.

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* We are grateful to Parke Davis & Co. for a generous supply of Antuitrin "S".

days after castration; 26 rats, 36 days after castration; 6 rats, 27 days after castration.

To facilitate graphic presentation of the data, the weight of the gland in milligrams was divided by the weight of the rat in grams and this factor multiplied by a constant. The result was designated as F K.

There was a slight variation in the experimental group as a result of variation of the dosage. This was not marked but the tendency seemed to indicate that the increase in weight of pituitary and adrenals is not in direct proportion to the dose.

The pituitaries were increased in weight 29.7%, the adrenals 37.5%, and the thyroid 8.4% as compared with the non-injected control group.

Conclusion. Antuitrin "S" increased the weights of the pituitaries, adrenals and thyroid in castrate rats.

6972 C

Effect of Theelol on Weights of Pituitary, Adrenal and Thyroid.

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Theelol* and Theelin* differ in their quantitative estrogenic action, in their chemical structure and in their absorbability from the human intestine.[1, 2] The morphological changes, as evidenced by organ weights, produced by estrin in the form of Amniotin (E. R. Squibb & Co.) have been studied by Leonard, Meyer and Hisaw.[3] No such data is available for Theelol.

We present the variations in weights of the pituitary, thyroid and adrenal glands following injections of Theelol.

Eighteen sexually mature albino rats weighing between 160 to 220 gm. were oophorectomized. Twenty-two days were allowed to intervene before injections were begun. Theelol was furnished in

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* We are grateful to Parke Davis & Co. for a generous supply of Theelol and Theelin.

[1] Thayer, S. A., Levin, L., and Doisy, E. A., *J. Biol. Chem.*, 1931, **91**, 655.

[2] Mazer, C., and Goldstein, L., Clinical Endocrinology of the Female. W. B. Saunders Co., Phila., 1932.

[3] Leonard, S. L., Meyer, R. K., Hisaw, F. L., *Endocrinology*, 1931, **15**, 17.

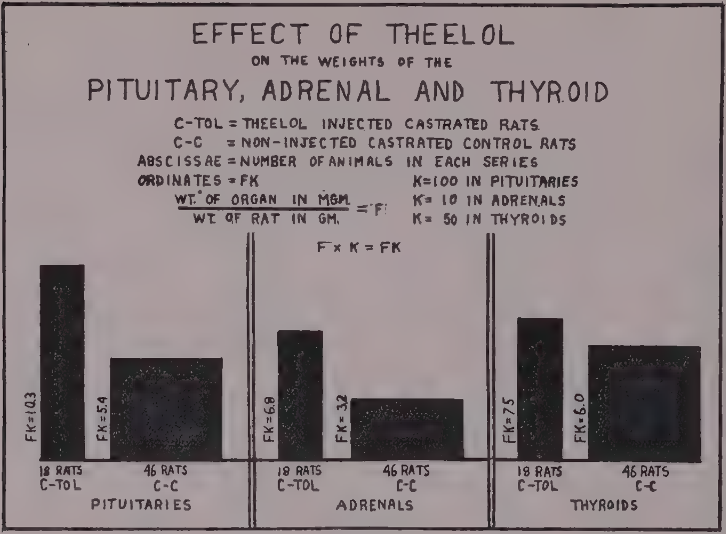

FIG. 1.

tablets containing 2 mg. of active substance, equivalent to approximately 100 rat units. Each rat received 2 tablets daily for 6 days. The tablets were dissolved in 6 cc. of sterile distilled water and injected subcutaneously.

At the end of the period of injection, *i. e.*, the 28th day after castration, the animals were sacrificed. Their glands were removed and weighed on a torsion balance.

Forty-six sexually mature animals weighing between 140 to 269 gm. were sacrificed in 3 groups: 6 rats on the 27th day after castration; 14 rats on the 28th day after castration; 26 rats on the 36th day after castration.

Because of the variation in the weights of the animals and because organ weights vary to a great degree with total animal weights, it was thought desirable to express the organ weights in terms of milligrams of gland per gram of animal weight. This factor was multiplied by an appropriate constant to facilitate plotting on a chart for the purpose of comparison. The factor multiplied by the constant is designated as F K. We believe this gives a more graphic presentation of the data. It is realized that this might give a false impression if the animals had lost a significant amount of weight during the course of the experiment. This, however, was not the case.

In the experimental series there was an increase in weight of

91% for the pituitaries, 112.5% for the adrenals, and 25% for the thyroids, when compared with the control series.

The significant histological findings of the various organs will be presented at a later date.

Conclusion. Injections of Theelol cause increase in the weights of the pituitary, adrenal and thyroid glands as compared with the controls.

6973 C

Combined Effect of Antuitrin "S" and Theelin on Weights of Pituitary, Adrenal and Thyroid in the Normal Rat.

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From the Department of Obstetrics and Gynecology, Vanderbilt University School of Medicine, Nashville, Tenn.

Urinary hebin (Antuitrin "S")* and estrin (Theelin)* are found in large amounts in the human pregnant urine.[1, 2] In studying the effect of these substances it became apparent that significant variations in organ weight occurred as a result of the injections of a mixture of the substance.

Twenty-two normal adult albino rats were injected with a mixture of 2 rat units of Antuitrin "S" and 20 rat units of Theelin daily for 9 days. A further group of 22 normal albino rats received 2 rat units of Antuitrin "S" daily for 9 days. Thirty-two non-injected normal adult albino rats were used as controls. At the end of the injection period all the animals were sacrificed and the glands weighed on a torsion balance.

The weight of the animals in the control series varied from 140 gm. to 220 gm., and the pituitaries from 6.5 mg. to 17.5 mg. In the normal animals, injected with Antuitrin "S", the body weight varied from 116 gm. to 140 gm., and the pituitary weights from 8 mg. to 12 mg. In the group injected with Antuitrin and Theelin, the body weights ranged from 120 gm. to 152 gm. and the pituitary weights from 7.5 mg. to 15 mg. The results are expressed in terms of milligrams of gland per gram of rat. This index was multiplied by an appropriate constant to facilitate graphing, and is designated as F K.

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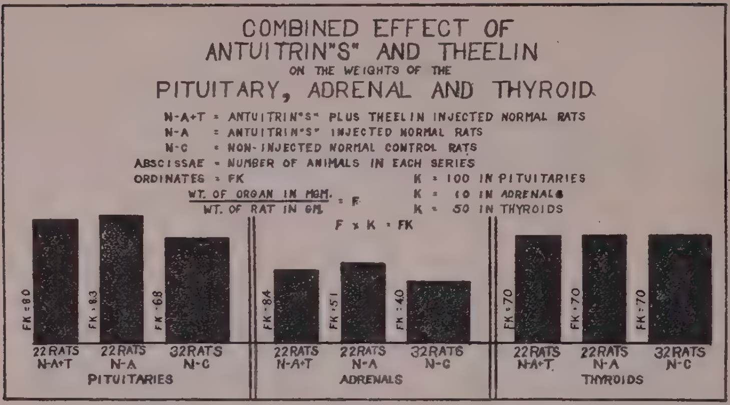
* We are grateful to Parke Davis & Co. for a generous supply.

[1] Aschheim, S., Zondek, B., *Klin. Wchnschr.*, 1928, **7**, 1401.

[2] Zondek, B., *Klin. Wchnschr.*, 1928, **7**, 1404.



Resulting from the injections of 2 rat units of Antuitrin "S", the pituitaries were increased in weight 22% and the adrenals 27.5%, as compared with the normal control animals. Whereas, the injections of 2 rat units of Antuitrin plus 20 rat units of Theelin only caused an increase of 17.7% for the pituitaries and 20% for the adrenals as compared with the normal control rats. No significant changes in the weights of the thyroids were found.

Conclusion. 1. A combination of Antuitrin "S" and Theelin increases the weight of the pituitary and adrenal in the normal rat. 2. Antuitrin "S" alone causes a slightly greater increase than Antuitrin "S" plus Theelin.

6974 C

Lipolytic Activity of Rat Lepromata During Treatment with Various Antileprotics.*

GEORGE EMERSON, H. H. ANDERSON AND C. D. LEAKE.

From the Pharmacological Laboratory, University of California Medical School, San Francisco.

We previously discussed the distribution of lipase in tissues of normal and experimentally infected leprous rats,[1] as observed by us preliminary to a study of changes in lipolytic activity during treatment of infected animals. Our subsequent observations demonstrating that there is no significant change in lipolytic activity of tissues of leprous animals during treatment are reported elsewhere.[2] This present report is concerned with changes in the lipase content of the leproma during intensive "plancha" treatment[3] with various antileprotic drugs.

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* Part of a cooperative study of the chemotherapy of leprosy conducted by the Hooper Foundation for Medical Research and the Pharmacological Laboratory of the University of California Medical School, San Francisco, and supported in part by the Christine Breon Fund for Medical Research.

The lipolytic activity of 60 untreated lepromata and 36 treated lepromata was estimated by Loevenhart's method[4] as used by us previously, and was found to be 0.16±0.03% and 0.15±0.04% respectively. Approximately half the material in both cases was obtained by biopsy and the other half from animals dying or sacrificed. It is apparent from these determinations that there is no tendency during treatment for the lipolytic activity of lepromatous subcutaneous tissue to approach the value of 0.83±0.07% found by us for normal subcutaneous tissue of rats with "early stage" leprosy.

No correlation was found between apparent lipase content of lepromata and type of antileprotic drug used. The average lipolytic activity of lepromata of rats treated over 6 months with maximum tolerated doses of the more important drugs considered was: ethyl chaulmoograte, 0.15±0.03%; "Alepol," 0.15±0.05%; ethyl di-n-heptyl acetate,† 0.11±0.04%; sodium dihydrochaulmoogryl p-phenetidine sulfonate, 0.12±0.05%; sodium chaulmoogryl p-phenetidine sulfonate, 0.13±0.02%.

Two possible explanations of the cause of the low lipolytic activity of leprous tissues were examined experimentally. First, the absence of any lipase-inhibitory substance in lepromata was shown by the addition of leproma brie to other tissue extracts which results in a summation of lipolytic activity, even if both are incubated together for a short time. Secondly, mechanical dilution of tissue by the leprosy bacillus, which has a low lipase content, was considered.

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[1] Emerson, G., Anderson, H. H., and Leake, C. D., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 150.

[2] Anderson, H. H., Emerson, G., and Leake, C. D., *Am. J. Trop. Med.*, 1933, in press.

[3] Lara, C. B., and Nicolas, C., *J. Philip. Is. Med. Assn.*, 1929, **9**, 321; Velasco, F. I., Alonso, J. M., Limkako, G., and Fernandez, G., *Ibid.*, 1929, **9**, 327; Lara, C. B., *Ibid.*, 1929, **9**, 336.

[4] Kastle, J. H., and Loevenhart, A. S., *Am. Chem. J.*, 1900, **24**, 491; Loevenhart, A. S., *Am. J. Physiol.*, 1902, **6**, 331.

† We are indebted to Dr. Roger Adams, Department of Chemistry, University of Illinois, for kindly furnishing us with a quantity of di-n-heptyl acetic acid, and to Dr. Richard Wrenshall, Department of Chemistry, University of Hawaii, for generously supplying us with various new chaulmoogryl derivatives as well as with ethyl chaulmoograte.

Determination of the ethyl butyrate esterase content of *Mycobacterium leprae hominis* (Mary Puhulahula strain, isolated in pure culture by Dr. E. L. Walker) gave values of 0.27 to 0.28% for a 10% suspension on duplicate analyses. Kendall, Walker and Day[5] have also reported extremely low values for the human leprosy bacillus. Since this organism is not the pathogen of rat leprosy, these results have no exact relation to the values found for lepromata, which are themselves nearly pure cultures of *M. leprae muris.* They give a strong indication of the lack of lipase in the rat leprosy bacillus, however. It is unfortunate that *M. leprae muris* cannot be cultivated *in vitro* sufficiently well to make possible a similar study with it (Koch[6]).

It is concluded, therefore, that the apparent low lipase content of lepromata is real, and that this condition is probably brought about by the dilution of normal tissue by tremendous numbers of *M. leprae.*‡ This holds for other tissues of infected rats as well, since massive invasion of most internal organs may occur (Schlossberger and Koch[7]). The explanation of the wide variation of lipolytic activity previously found is obvious. Also, none of the drugs studied produces lipase activation *in vivo,*§ confirming the work of Neill and Dewar[8] with ethyl chaulmoograte in human lepers. In view of the failure of leprosy therapy with a specific lipase activator ("javanin," Eubanas[9]) and the reputed clinical efficacy of chaulmoogra derivatives, it is doubtful that the latter act therapeutically through an indirect stimulation of the lipolytic activity of the tissues, as suggested by Shaw-Mackenzie and Rogers.[10]

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[5] Kendall, A. I., Walker, A. W., and Day, A. A., *J. Inf. Dis.*, 1914, **15**, 443, 468.

[6] Koch, F., *Z. f. Haut- u. Geschlechtskrankh.*, (orig.), 1932, **40**, 433.

‡ A somewhat similar explanation of the cause of the apparent low lipolytic activity of tubercular guinea pig tissues has been proposed. Happold, F. C., and Taylor, A., *Brit. J. Exp. Path.*, 1927, **8**, 101; Susman, W., and Happold, F. C., *Ibid.*, 1927, **8**, 106; Happold, F. C., and Taylor, A., *Ibid.*, 1931, **12**, 272.

[7] Schlossberger, H., and Koch, F., *Z. f. Bakt., Parasitenkunde u. Infektionskrankh (Sitzber.)*, 1932, **106**, 382; Schlossberger, H., and Koch, F., *Gedenkschrift f. Joannovic*, Belgrad, 1932.

§ We have been unable to confirm Shaw-Mackenzie's report of *in vitro* activation, also. Further work on this is in progress.

[8] Neill, M. H., and Dewar, M. M., *U. S. P. H. Service Pub. Health Bull.*, 1927, **168**, 21.

[9] Eubanas, F. C., *J. Philip. Is. Med. Assn.*, 1927, **7**, 407; Pock-Steen, P. H., and Tuxen, G. E., *Acta pathol. microbiol. Scandinav.*, 1926, **3**, 681.

[10] Shaw-Mackenzie, J. A., *J. Trop. Med. Hyg.*, 1921, **24**, 161; Rogers, L., *Brit. Med. J.*, 1923, **2**, 11; *Lancet*, June 28, 1924, 1297.

6975 P

Peculiarities of the Prolan-like Substance in Urine in a Case of Embryonal Carcinoma of the Testis.

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Extensive biological study has been made of the gonadotropic substance, prolan, which occurs in the urine of pregnant women. Unlike the gonadotropic hormone occurring in the anterior hypophysis itself, prolan even when concentrated, is sharply limited[1] in its capacity to cause development of the ovaries in immature rats. When combined with a special synergic principle of the anterior hypophysis,[2, 3] a marked augmentation of the ovary weights occurs. The gonadotropic effects are then fully equal to those produced by the most potent extracts of the gland itself.

In the study of the synergism phenomenon with prolan-like substances from sources other than pregnancy, the urines of several types of malignancy were investigated. Among these cases was an individual suffering from embryonal carcinoma of the testis with generalized metastases. Ferguson[4] has shown the remarkably high prolan unitage in the urine of such cases.

The urine in question had an extraordinarily high unitage (50,000 mouse units per liter). In addition, the prolan-like substance from this patient possessed other unusual characteristics. It gave a remarkable stimulation to ovarian development with *no* demonstration of the limited effect characteristic of pregnancy-prolan. Indeed, this urinary product could be classified (in ability to give ovarian stimulation) with the most potent gonadotropic substances known, but like pregnancy prolan, the substance retained its ability to give augmentation of ovary weights when combined with the hypophyseal synergic principle. When the urinary product in question was tested in males there was a tremendous development of the seminal vesicles —much greater than shown by pregnancy-prolan. Table I summarizes the properties of this unusual urinary product as tested in immature male and female rats and compares its potency with pregnancy-prolan.

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[1] Evans, H. M., Meyer, K., and Simpson, M. E., *Am. J. Physiol.*, 1932, **100**, 141.

[2] Evans, H. M., Simpson, M. E., and Austin, P. R., *J. Exp. Med.*, 1933, **57**, 897.

[3] Evans, H. M., Simpson, M. E., and Austin, P. R., *J. Exp. Med.*, 1933, in press.

[4] Ferguson, R. S., *Am. J. Cancer*, 1933, **18**, 269.

TABLE I
Comparison of Pregnancy Prolan with the Urinary Product from a Case of Embryonal Carcinoma of the Testes

Gonadotropic substance			Combination with synergic factor* (*in vitro*)		Male rats		
Source	Total Dose mg.	Wt. ovaries mg.	Wt. ovaries mg.	Activation %	Total dose mg.	Wt. seminal vesicles mg.	Wt. testes mg.
Urine of pregnancy	0.14 (Infantile)	16	32	45	109 (12 days)	85	610
	0.54	26	33	10			
	2.73	26	108	260			
	13.6	38	130	210	(Controls)	(6)	(492)
	27.3	38	187	345			
	81.8	49	209	294			
Urine from case of embryonal carcinoma of testes with generalized metastases	0.14	30	93	173	45 (10 days)	185	861
	0.54	47	167	227			
	2.73	60	186	190	(Controls)	(9)	(446)
	13.6	68	176	144			
	54.4	220	192	0			

*Aqueous alcohol extract of pig anterior hypophysis: dose 1.36 mg.; weight of ovaries, 22 mg. The given weights of prolan and synergic factor were mixed *in vitro* and injected subcutaneously, daily, on 3 successive days with autopsy after 96 hours [2], [3]. Three rats were used at each dilution.

Riddle and co-workers have shown convincingly that the bird testis is peculiarly insensitive to prolan.[5] Some of this special urinary material was sent to Dr. Oscar Riddle for assay and he has reported this to be the first urinary product tested by him that stimulated development of the bird's testis. Sacrifice of the 2 doves employed by him after 8 days of treatment showed increases of 400 and 800% respectively in the weight of the testes. (Table II.)

TABLE II
Assay in Immature Ring Doves of Gonadotropic Hormone in Urine from the Case of Embryonal Carcinoma of the Testis.

Bird No.	Age mo.	Body Wt. gm.	Total Dose mg.	Treatment days	Wt. of Testes Exper. mg.	Wt. of Testes Control mg.
1	2.3	155	60	8	79.3	6.8*
2	2.3	132	60	8	46.6	6.8

*Average of testes of 10 controls with a range of 4.0 to 10.0 mg.

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[5] Riddle, O., and Polhemus, I., *Am. J. Physiol.*, 1931, **88**, 121.

We are exploring other cases of high unitage of urinary prolan (*e. g.*, chorioepithelioma) to see if they also are characterized by the excretion of this peculiar type of gonadotropic substance.

6976 P

Effect of Radiant Energy With and Without Iron Upon Nutritional Anemia in the Rat.

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The effect of radiant energy upon the metabolism of small doses of Fe in the nutritional anemia of the rat seems to have been little studied. Foster[1] produced anemia in rats by milk feeding and observed the effect of radiant energy in preventing and curing this type of anemia. There was a slight but definite effect in increasing the hemoglobin and the number, size and saturation of the red cells.

We have made the following studies upon the effect of radiant energy upon both the prevention and cure of nutritional anemia in the rat.

Preventive Studies. Young rats at weaning were fed upon whole milk. Daily doses of pure Fe from 0.05 to 0.30 mg. were given, with and without ¾ hour ultraviolet irradiation of the rat daily, with the General Electric Sunlight Mazda Lamp, Type S-I, at a distance of 4 feet. This lamp has recently been described by Carter.[2] Weekly estimations of hemoglobin and red cell counts were made by the technique described by Beard and Myers.[3]

With milk alone, milk plus irradiation, irradiated milk, and milk plus 0.05 mg. Fe daily the anemia became progressively worse. There was a striking effect of irradiation on red cell recovery with both 0.05 and 0.10 mg. Fe daily, namely, a drop of 41 and 0.6% on these doses of iron may be compared to an *increase* of 33 and 50% when ¾ hour irradiation was given. The anemia was prevented by daily doses of Fe from 0.15 to 0.30 mg. These doses with irradiation were also much more effective in preventing the anemia than were these doses of Fe alone, with the exception of the 0.25 mg. dose. Irradiated milk with 0.30 mg. Fe daily gave a much more striking increase in both cells and hemoglobin than any other type of therapy used in this study. These results are given in Table I.

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[1] Foster, P. C., *J. Nutr.*, 1932, **4**, 517.

[2] Carter, H. A., *J. Am. Med. Assn.*, 1932, **99**, 31.

[3] Beard, H. H., and Myers, V. C., *J. Biol. Chem.*, 1931, **94**, 71.

A polycythemia occurred in 4 animals on doses of Fe alone and in 12 animals on Fe and irradiation.

Curative Studies. At the end of 6-8 weeks milk feeding, the anemic young rats were given the same treatment outlined above. The most marked effect of the irradiation on red cell and hemoglobin recovery was again demonstrated with 0.05 mg. Fe daily. But the irradiation with doses of Fe from 0.10 to 0.30 mg. had no better effect upon hemoglobin recovery than these doses of Fe alone, with the exception of the 0.25 mg. group. Hemoglobin values of 8-11 gm. per 100 cc. were obtained in all these groups in 7-10 weeks which was somewhat slower than we have previously reported. Red cell recovery was excellent in all these groups and was more marked with 0.15, 0.20 and 0.30 mg. Fe daily than it was with these doses of Fe alone. (Table II.)

A polycythemia occurred in 9 cases with Fe alone and in 22 cases on Fe with irradiation, being most pronounced with 0.20 mg. Fe than with any other dose. In some animals this polycythemia was still present at the end of the 10 weeks experimental period and in others a normal cell count of 8-9 m/cu.mm. occurred in about 2 weeks after the highest values were obtained.

A total of 224 animals were used in these studies and in a large number of them a daily milk intake above 50 cc. was rarely seen.

It is evident that the beneficial effect of irradiation of the rat and the milk observed is due to the formation of vitamin D under these conditions. To test this point further 2 anemic rats were given 0.30 mg. Fe daily with the addition of 1 drop of a potent vitamin D preparation added daily to the milk. The results were as follows:

	Hemoglobin gm./100cc.		R.B.C. m/cu.mm.		Time of recovery Weeks	
Rat. No.	Before	After	Before	After	Hb	R.B.C.
1177	3.0	15.4	3.99	7.42	6	2
1178	5.0	14.3	3.80	7.42	6	3

Evidently our milk was lacking in vitamin D and when this was added, either by irradiating the rat, milk, or adding vitamin D to the milk, hemoglobin and red cell recovery took place in exactly the same time as occurred in our previous studies. We believe that the somewhat slower responses in red cells and hemoglobin observed in the present studies were due, not to a need for Cu, but rather to a

TABLE I
Summary of Prevention Experiments.

	Aver. % change			Time of Recovery Weeks		Diet
No. Rats	Body Wt.	Hb.	R.B.C.	Hb.	R.B.C.	
6	+112	–63	–51	5	5	Milk alone.
9	+118	–51	–39	5	5	Irrad. milk
13	+109	–54	–39	5	5	Milk + ¾ hr. irrad.daily
5	+190	–49	–41	7	7	Milk + .05 mg. Fe daily
9	+290	–18	+33	10	10	Milk + .05 mg. Fe + ¾ hr. irrad. daily.
4	+227	–0.4	–0.7	10	10	Milk + .1 mg. Fe daily
9	+295	–9.0	+49	10	10	Milk + .1 mg. Fe daily + ¾ hr. irrad. daily.
5	+354	+ 8	+62	10	10	Milk + .15 mg. Fe daily.
10	+420	+15	+92	10	10	Milk + .15 mg. Fe + ¾ hr. irrad. daily.
5	+509	+18	+51	10	10	Milk + .20 mg. Fe daily
10	+355	+23	+77	10	10	Milk + .20 mg. Fe + ¾ hr. irrad. daily.
5	+501	+44	+77	10	10	Milk + .25 mg. Fe daily
10	+452	+34	+60	10	10	Milk + .25 mg. Fe + ¾ hr. irrad. daily.
5	+311	+17	+49	10	10	Milk + .3 mg. Fe daily
7	+331	+26	+80	10	10	Milk + .3 mg. Fe + ¾ hr. irrad. daily.
10	+502	+94	+109	10	10	Irrad. milk + .3 mg. Fe daily.

TABLE II
Summary of Curative Experiments.

	Average % change in			Time of Recovery Weeks		
No. Rats	Body Wt.	Hb.	R.B.C.	Hb.	R.B.C.	Diet
10	+74	–2	+23	9	9	Milk alone.
12	–1	–14	–11	5	5	Milk + ¾ hr. irrad. daily.
5	+61	+6	+13	4	4	Milk + .05 mg. Fe daily.
9	+121	+69	+85	6	8	Milk + .05 mg. Fe + ¾ hr. irrad. daily.
3	+170	+161	+273	9	8	Milk + .1 mg. Fe daily.
7	+93	+139	+194	7	9	Milk + .1 mg. Fe daily + ¾ hr. irrad. daily.
6	+194	+146	+187	7	8	Milk + .15 mg. Fe daily.
8	+82	+83	+221	7	8	Milk + .15 mg. Fe + ¾ hr. irrad. daily.
6	+180	+229	+287	9	9	Milk + .2 mg. Fe daily.
6	+78	+223	+344	8	7	Milk + .2 mg. Fe + ¾ hr. irrad. daily.
4	+176	+151	+272	9	8	Milk + .25 mg. Fe daily.
10	+86	+233	+276	7	7	Milk + .25 mg. Fe + ¾ hr. irrad. daily.
5	+227	+248	+238	8	8	Milk + .3 mg. Fe daily.
11	+172	+180	+273	7	8	Milk + .3 mg. Fe + ¾ hr. irrad. daily.

need of vitamin D with Fe. This again confirms our view that the nutritive value of the milk and its daily quantitative intake are factors of importance in Fe metabolism in studies of nutritional anemia in the rat.

We have data to show that the above effects were not due to the Cu content of either our milk or Fe solutions. It would seem that negative reports of the effect of Fe alone upon hemoglobin recovery in anemia studies would be of limited value unless definite assurance is given that the vitamin D content of the milk used is adequate. Work along this line is in progress.

6977 C

Relative Inagglutinability of the Castellani-Sonne Group of Dysentery Bacilli.

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During the summers of 1931 and 1932 five strains of the Castellani-Sonne or Thjøtta type III dysentery bacilli were isolated at the Babies and Childrens Hospital from the stools of 5 children under 5 years of age. All the patients showed a typical course of dysentery infection. Among them one death occurred. It is emphasized here that increasing proof of the prevalence of this type of dysentery apparently is forthcoming. Especial attention should, therefore, be given to the isolation and identification of this type organism.

The organisms were minute, non-motile, gram negative rods, readily acidifying glucose, mannite, maltose, and rhamnose. Lactose was slowly acidified (one to 2 weeks) and the same was true for sucrose. Litmus milk was acidified by all strains and coagulated by 4 strains. Indole was not formed. In broth, marked sedimentation occurred, but there was no pellicle formation. The cultural characteristics of these 5 strains closely corresponded to those observed in 2 typical strains, No. 268 and Sonne B, obtained through the courtesy of Dr. S. A. Koser. Three out of 5 of our strains and the 2 Koser strains clotted milk in 21 days. The same basic reactions were described by Wiseman,[1] Fyfe,[2] Braun and Weil,[3]

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[1] Wiseman, W. R., *Lancet*, 1927, **1**, 817; *J. Hyg.*, 1927, **26**, 187.

[2] Fyfe, G. M., *J. Hyg.*, 1927, **26**, 271.

[3] Braun, H., and Weil, A. J., *Centralbl. f. Bakteriol.* (Abt. 1), 1928, **109**, 16.

Koser, Reiter, Bortniker and Swingle,[4] and Soule and Heyman.[5] Rhein,[6] however, failed to find acidification of lactose but this may be accounted for on the basis of insufficient incubation time.

Polyvalent antidysentery sera failed to agglutinate the 5 strains. All agglutinations were performed by incubation in a water bath at 37°C. for 2 hours and overnight in an icebox at 4 to 5°C. The polyvalent sera also failed to agglutinate the new strains by the centrifuge method. It was then decided to immunize rabbits with these strains in order to determine their agglutinability in the presence of homologous sera.

Three rabbits received massive doses of killed and live organisms (a total of four 24-hour slants of pooled strains for each rabbit) over a period of 6 months. Sixteen injections were given, 5 with killed bacteria (heated at 60°C. for from one to 2 hours) and 11 with live organisms. The first 10 doses were injected by the intravenous route; the remaining injections were given intravenously, intraperitoneally, and subcutaneously. During this period of injections, 5 bleedings were made, but the sera failed to agglutinate the organisms when agglutinations were performed in a water bath at 37°C. for 2 hours, or at 56°C. for 4 hours according to Koser, and the suspensions allowed to stand for 24 hours at icebox temperature (4 to 5°C.). The sera also failed to clump Flexner strains W. V, and Y. Complement fixations, however, employing the usual antisheep hemolytic system, 2 units of complement, and the 5 strains as antigens, gave the following results: Serum No. 203 gave fixation in as small a dose as 0.0001 cc., serum No. 201 in 0.0002 cc., and serum No. 202 in 0.0004 cc. doses.

It is well known that freshly isolated strains of this organism often fail to agglutinate (Wiseman,[1] Fyfe.[2]). However, the 5 strains have undergone numerous transplantations during the course of almost 2 years and they still fail to agglutinate. Koser[4] further states that it was impossible to prepare satisfactory sera in certain rabbits, although in others satisfactory sera were obtained.

Since all agglutinations failed, it was thought of interest to increase cohesion of the organisms by centrifugation. Serum No. 203 gave complete agglutination in a dilution of 1:640. All the 7 strains including the 2 Koser strains clumped, whereas the control tubes failed to show clumping on resuspension of the organisms. Serum

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[4] Koser, S. A., Reiter, D. O., Bortniker, E., and Swingle, E. L., *J. Prev. Med.*, 1930, **4**, 477.

[5] Soule, M. H., and Heyman, A. M., *J. Lab. and Clin. Med.*, 1933, **18**, 549.

[6] Rhein, M., *Compt. rend. Soc. de biol.*, 1933, **112**, 814.

No. 202 gave the same result for 3 strains, but clumping was less marked for the 4 others. Serum No. 201 agglutinated one strain up to 1:640, four others agglutinated from 1:40 to 1:80, and 2 remained negative. Active sera gave larger clumps than inactivated sera.

Summary. Five strains of Castellani-Sonne dysentery bacilli were isolated at the Babies and Childrens Hospital during the summers of 1931 and 1932. The organisms, in so far as they were studied, were culturally and biochemically typical, but they were inagglutinable by their homologous antisera when agglutinations were performed in water baths at 37° and 56°C. and at icebox temperature overnight. However, by centrifugation of the suspension, 2 sera clumped all the 7 strains, while the third serum gave less marked clumping. Complement fixation gave excellent results, and doses of the sera as small as from 0.0001 to 0.0004 cc. fixed the complement in the presence of the strains. However, it is suggested that the centrifuge method of agglutination be used for routine diagnosis of this group of organisms on account of the slow appearance of the differentiating biological characters of the group and because of the relative inagglutinability of the strains by the usual method of agglutination.

6978 C

Effect of Adenylic Acid on Gastric Secretion.*

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Following the isolation of histamine from the pyloric mucosa,[1] it was suggested that histamine might be found in the blood stream after the ingestion of a meat meal. The extraction of quantities of blood for histamine by various methods gave a substance which possessed vasodepressor properties and gave a positive Pauly reaction but was uniformly negative (subcutaneous) in producing a gastric response.

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* This investigation has been aided by a grant from the Josiah Macy, Jr. Foundation.

[1] Sacks, J., Ivy, A. C., Burgess, J. P., and Vandolah, J., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 941.

Adenylic acid is one of the vasodepressor substances found in the blood stream.[2] According to Bennet and Drury,[3] 3 mg. of adenylic acid intravenously cause a blood pressure fall of 40 mg. of mercury. Believing that this substance might be capable of producing a gastric secretory response, doses from 1.0 to 10.0 mg. were injected subcutaneously into Pavlov pouch dogs. The gastric response was uniformly negative.

6979 P

Cyclic Variations in Urinary Excretion of Iodine in Women.*

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While investigating the daily urinary excretion of iodine in patients with goiter, we observed a rise in the urinary output of iodine during the first 2 days of menstruation in one (Fig. 1, B), with non-toxic nodular goiter, and a premenstrual rise in the other (Fig. 1, A), with exophthalmic goiter. Several investigators have reported an increase in the blood iodine during menstruation.[1, 2, 3, 4] It has also been reported that menstrual blood is considerably higher in iodine content than venous blood.[3, 5]

Since the urine was not obtained by catheterization some unavoidable contamination occurred. However, 50 to 100 cc. of menstrual blood would be required to give the increase found during the menstrual period in the patient (B), and blood was not grossly evident in the specimens. To further investigate this cyclic variation, 2 women without evidence of thyroid disease (C and D) were studied throughout the entire menstrual cycle.

During these studies the subjects were allowed a general diet. Care was taken to exclude foods known to be high in iodine content, and particularly sea foods. The ages are as follows: A-18, B-39,

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[2] Zipf, K., *Arch. exp. Path. u. Pharm.*, 1931, **160**, 579.

[3] Bennet, D. W., and Drury, A. N., *J. Physiol.*, 1931, **72**, 288.

* This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

[1] Maurer, F. E., *Archiv. f. Gynäk.*, 1927, **130**, 70.

[2] Jahn, D., and Kesselkaul, O., *Deutches Arch. f. klin. Med.*, 1928, **161**, 143.

[3] Kesselkaul, O., *Verhandl. d. Gesellsch. f. Inn. Med.*, 1928, **40**, 294.

[4] Scheringer, W., *Arch. f. Gynäk.*, 1930, **143**, 319.

[5] Bourcet, P., *Compt. rend. Acad. d. sc.*, 1900, **131**, 392.

C-27, and D-32. All 4 showed either a premenstrual or menstrual rise in urinary iodine excretion (Fig. 1). Subject C showed the premenstrual rise during 2 consecutive periods. B, C, and D showed a rise 6 to 8 days after the onset of menstruation. A, C, and D presented a rise 14 to 18 days after the onset of menstruation.

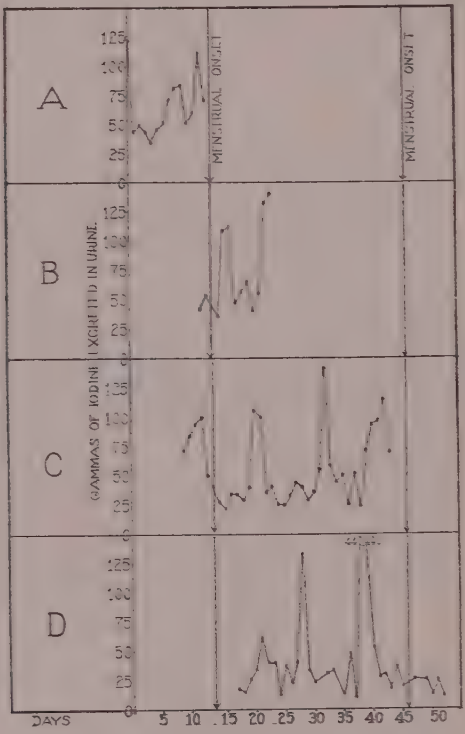

Fig 1 DAILY URINARY EXCRETION OF IODINE IN WOMEN

It will be noted that the variation in the urinary iodine excretion does not parallel that found in the blood iodine by previous workers. At present we are not prepared to discuss the mechanism of this cyclic variation in the urinary excretion of iodine. Further investigation is in progress.

6980

A Second Note on the Communicability of Colds.*

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It is generally accepted that the common cold is extremely contagious during the primary stage of the affection, and recent observations[1] have tended to show that the common cold is transmissible during its incubation period. Undoubtedly the spread of the contagion from individual to individual during these two stages accounts for the majority of colds, but there are numerous instances of infection in which this mode of spread cannot be substantiated.

The possibility that the infectious agent may be spread by food or fomites has always had many adherents, but as yet there have been no carefully controlled observations upon this point. We, therefore, decided to put to experimental test the belief that the infectious agent of colds can be transmitted upon food contaminated by an individual suffering from an acute common cold.

At the time of the experiment 15 chimpanzees had been under constant observation from July 15 until September 19, 1932. None had shown any signs of a cold. On September 19 the animals were placed in strict isolation in separate cages, distributed in 4 widely separated rooms and during the isolation period the doors to these rooms were locked. The attendants wore masks and gowns and scrubbed their hands thoroughly before coming in contact with the apes or their food. All of the attendants were examined daily during the entire period of isolation for signs of upper respiratory tract infection. All remained healthy. The isolation technique was continued for a period of 3 weeks.

Upon September 26 (the seventh day of isolation) an individual suffering from an acute common cold (second day) prepared breakfast, luncheon and supper for the apes. This individual saw neither the apes nor the attendants while entering the kitchen or preparing the food. After the food was prepared and placed in the containers the individual ill with the cold departed and the attendants then placed the food in the apes' cages.

Within 48 hours after the first meal, 5 of the apes had developed

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* This investigation was supported by a grant from the John J. Abel Fund for Research on the Common Cold, The Johns Hopkins University.

[1] Long, P. H., Bliss, E. A., Carpenter, H. M., *Bull. Johns Hopkins Hosp.*, 1932, **51**, 278.

typical common colds characterized by nasal discharge, nasal obstruction, mouth breathing, slight fever, and a leukocytosis. Two of these apes developed moderately severe coughs. The nasal discharge persisted in all animals for at least 10 days. There were no secondary cases among the other 10 apes.

Summary. We feel that the observations just recorded add experimental proof to the common belief that colds may be spread by infected food, and bring into the foreground the necessity of excellent personal hygiene upon the part of those individuals ill with common colds.

6981 P

Metabolism in Pregnancy: XI. Blood Sugar Changes During Delivery.

ALLAN WINTER ROWE AND MARY MCMANUS.

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In the course of an extended investigation on various aspects of the metabolism during pregnancy a series of records were made while the patient was actually in labor and immediately thereafter. It is a well-recognized fact that during pregnancy blood sugars are at a low normal level. In our own series[1] the average level throughout gestation was 83 mg. and the average extreme fluctuation from 81 to 86 mg. In the present instance, a series of 25 patients were exam-

TABLE I
General Data

	Average or Record
Age, high	40 yrs.
low	18 ”
average	25 ”
Parity, high	10
low	1
average	2.8
Duration of Labor, high	41 hr. 55 min.
low	0 ” 55 ”
average	12 ” 54 ”
Last food before a.p. blood, high	21 hr. 30 min.
low	0 ” 45 ”
average	11 ” 2 ”
Time of a.p. blood before delivery, high	27 hr. 25 min.
low	0 ” 15 ”
average	6 ” 4 ”

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[1] Rowe, Gallivan and Matthews, *Am. J. Physiol.*, 1931, 96, 94.

ined at some time after labor had begun and blood was taken again 30 minutes after delivery. Certain details may be presented in tabular form.

Even so small a series presents a wide scatter of performance. There were 11 primiparae, and while the time in labor (16 hr. 32 min.) averages well above the mean for the group, the longest labor was the woman terminating her tenth pregnancy. The influence of nourishment was seemingly negligible. In the case receiving food (cocoa, orange juice, muffin) 45 minutes before the blood sample was taken, the blood sugar level was 80 mg., and in only 5 cases was the elapsed time less than 6 hours with an average level of 82 mg. In the analysis of the elapsed time from blood taking to delivery, that with the longest was 87 mg. and the shortest 85. The blood sugar data follow:

TABLE II
Blood Sugar Levels

			Amount Mg. %
Blood Sugar,	**a.p.,**	**high**	**89**
		low	**65***
		average	**82**
Same,	**p.p.,**	**high**	**135**
		low	**86**
		average	**108**

***Next lowest is 76 and only 5 below 80.**

Only one case in the entire series failed to show an appreciable rise. This was a primipara 9 hours and 20 minutes in labor with blood taken 3 hours and 20 minutes before actual delivery. Food had not been taken for 17 hours before the veinpuncture. The levels both a.p. and p.p. were 86 mg.

Summary. 1. The low blood sugar levels characteristic of pregnancy obtain actually during labor and apparently to within a few minutes of delivery. 2. There is a marked rise in blood sugar on emptying the uterus, the average increase being slightly more than 30%, and in individual cases reaching low hyperglycaemic levels. 3. The time factor as such seemingly plays no part. In the case with blood taken 15 minutes before delivery, the level was 85 mg.; 45 minutes later it was 108 mg., an increase of 27%.

6982

Pertussis Toxin or Antigen.

JOHN A. TOOMEY AND JOSEPH E. MC CLELLAND.

From the Department of Pediatrics, Western Reserve University, and the Division of Contagious Diseases, City Hospital, Cleveland, Ohio.

Mishulow, Mowry and Scott[1] demonstrated that exotoxic filtrates could be obtained from whooping cough cultures. They planted their organisms on chocolate agar over which they had poured a small amount of horse serum-beef heart broth. The small amount of diluent made the harvest of toxin necessarily small. On the other hand, Craster and Smith[2] mentioned that there was a signal absence of toxin formation in planted cultures, and Riesenfeld[3] stated that there was no proof that this bacillus produced a toxin in ascitic broth. Besredka[4] isolated toxic substances termed endotoxins from the dried and powdered organisms.

In 1928, we found that pertussis bacillus produced a substance in culture growth, the exact nature of which was problematic. With the report of Mishulow, Mowry and Scott, we felt that further work should be done along this line. Since large amounts of toxin could not be practically produced on solid preparations, various liquid media were tried. We found that Pelouze's and Viteri's gonococcous medium slightly modified by omission of the agar could be used to advantage. The formula was as follows: 250 cc. of veal infusion, 80 cc. of brain infusion, 3.3 gm. of peptone, 1.6 gm. of NaCl, and 1.6 gm. of sodium phosphate. The medium had a pH of 7.2.

There are numerous protein factors in this medium, and when we tested out the unplanted control filtrates by intradermal injection of amounts of 0.1 cc., we found that humans gave positive reactions in a little less than 1% of the individuals tested and that in all instances veal was the reacting factor.

Of the 4 strains of the American Type Culture Collection experimented with, No. 778, a strain isolated by W. H. Park, gave us best results and was used in our subsequent work.

The bacillus was planted and grown arbitrarily for 10 to 14 days in the medium described. There was a rapid growth on the bottom of the flask after the fourth to the fifth day. By the ninth day, this growth had extended along the bottom and up the sides as a thick, tenaceous, white film. Colonies connected with the bottom of the flask by a thick, ropey strand grew on the surface of the media. After 14 days, the culture was a thick, stringy, mucilagenous mass which adhered firmly to the smooth glass and was delivered only as large masses of semi-gelatinous consistency.

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[1] Mishulow, Lucy, Mowry, Isabelle W., and Scott, Eleanor B., *J. Immunol.*, 1930, **10**, 227.

[2] Craster, Chas. V., and Smith, Ellis, *J. Med. Soc. New Jersey*, 1931, **28**, 236.

[3] Riesenfeld, Edwin A., *J. Am. Med. Assn.*, 1923, **80**, 158.

[4] Teissier, P., Reilly, J., Rivalier, E., and Cambessédès, H., *J. Physiol. et Path. Gen.*, 1929, **27**, 549.

After having been grown for an average of about 19 days, the material was passed through a few layers of gauze to separate the sticky part from the filtrate since direct passing through a Berkefeld filter was impracticable. The filtrate was then filtered through coarse paper to separate it from any remaining stringy filament. Either this procedure was followed or the mass was centrifuged at high speed for from 10 to 20 minutes and the supernatant fluid poured off. Even with high speed centrifugation over a long time, there was difficulty in throwing down all of the sticky mass. The filtrate obtained in either case was first passed through an N or a V Berkefeld filter, then through a W filter, tested for sterility, 0.2% tricresol was added and the material was then bottled. The medium takes on a peculiarly distinctive but undescribable odor. The pH did not change much, averaging 7.4 in 10 specimens.

Six hundred and eighty individuals were injected intradermally with 0.1 cc. of the filtrate. There was always a local skin reaction in 6 hours. In 502 of the individuals tested, the local reaction persisted for 24 hours, ranging from ½ to 2 inches in diameter and from faint to deep redness with induration. The highest dilution which gave a positive reaction was 0.1 cc. of a 1/50 dilution in saline obtained after 14 days of growth. This would indicate that there were approximately 500 skin test units of a toxic or antigenic element in each cubic centimeter.

Conclusion. Whooping cough bacillus when grown in the liquid medium as described, produced a substance which in the dosages used caused a local skin reaction in either 6 or 24 hours after injection in all the 680 individuals tested. Whether there is a specific fraction for whooping cough in this material has not been determined.

6983

Effects of Primary Shock on Cardiac Output and Blood Pressure.[*]

ALFRED BLALOCK.

From the Department of Surgery, Vanderbilt University, Nashville, Tenn.

In a previous study,[1] the effects of uncomplicated hemorrhage on cardiac output and blood pressure were determined. Repeated removal of blood was usually associated with a decline in the cardiac output from 30 to 50% below the normal level before a marked diminution in the blood pressure occurs. Johnson and Blalock[2] found that secondary shock as a result of trauma to muscles, trauma to the intestines or burns is associated with a definite decrease in the output of the heart before the blood pressure is altered appreciably. On the contrary, the introduction of histamine[2] causes an initial decline in the blood pressure followed by a decrease in the cardiac output. Following the removal of the adrenal glands,[3] the blood pressure usually declines before the cardiac output is altered. Trauma to the central nervous system[4] is usually associated with a simultaneous decline in both the cardiac output and blood pressure.

The alterations in the cardiac output and blood pressure in primary shock were determined in the present experiments in order to compare these changes with those observed in secondary shock.

Dogs were profoundly anesthetized by sodium barbital administered intravenously. The dosage was 0.3 gm. per kilo of body weight. The control determinations of the cardiac output and blood pressure were performed approximately 2 hours after injection of barbital. The anterior abdominal wall of the animal was then struck many blows in rapid succession with flat surface of a board. This procedure occupied approximately 2 minutes. Thirty to sixty seconds later, the cardiac output and blood pressure were again determined. If free hemorrhage into the peritoneal cavity resulted from the traumatization, the experiment was discarded.

A cannula that was placed in the femoral artery was used for the determination of the blood pressure. The cardiac output was computed by the Fick principle:

$$\frac{\text{Oxygen consumption per minute}}{\text{Arterial oxygen content minus venous oxygen content}} = \begin{array}{l}\text{No. cc. of blood}\\ \text{flowing through}\\ \text{lungs per min.}\end{array}$$

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[*] Aided by a grant from the Fluid Research Fund of Rockefeller Foundation.

[1] Blalock, Alfred, *Arch. Surg.*, 1927, **15**, 762.

[2] Johnson, G. S., and Blalock, Alfred, *Arch. Surg.*, 1931, **23**, 855.

[3] Blalock, Alfred, and Beard, J. W., *J. Lab. and Clin. Med.*, 1933, **18**, 941.

[4] Blalock, Alfred, and Bradburn, H. B., *Arch. Surg.*, 1929, **19**, 725.

A Benedict spirometer was employed in determining the oxygen consumption. Arterial blood was obtained from the femoral artery and mixed venous blood from the right side of the heart. The oxygen content of the blood was determined with the Van Slyke-Neill manometric apparatus.

Ten experiments were performed. In all, the striking of the abdomen was followed by a decline in the blood pressure. The drop in the mean arterial blood pressure in the different experiments varied from 38 to 75 mm. Hg. The decline in blood pressure was usually of short duration. Associated with the drop in blood pressure, the output of the heart increased slightly in 7 experiments and decreased in 3. The consumption of oxygen increased during and immediately following the traumatization in 9 of the 10 experiments. The arterio-venous oxygen difference increased in some instances and decreased in others. These alterations are quite different from those that are found in secondary shock.

6984 P

Specific Carbohydrate of Type I Pneumococcus.

ALWIN M. PAPPENHEIMER, JR., AND JOHN F. ENDERS.
(Introduced by Hans Zinsser.)

From the Department of Bacteriology and Immunology, Harvard University Medical School.

When a Type I pneumococcus antiserum is absorbed with the homologous soluble specific carbohydrate until no further precipitin reaction occurs with the latter, the absorbed serum still reacts type-specifically with a substance in pneumococcus autolysates in high dilution, as shown by Enders[1] and confirmed by Wadsworth and Brown[2, 3] and by Sabin.[4] We have now succeeded in isolating this substance, called the "A substance" by Enders, from Type I pneumococcus broth cultures.

The initial steps in the preparation of the A substance are essentially those given by Heidelberger and Kendall[5] in their preparation of the soluble specific substance, SSS1, except that care is taken to maintain an acid reaction throughout. When, as in their procedure, the specific carbohydrate is precipitated with acetic acid at pH 3.4, the A substance remains in solution. We have found, contrary to Heidelberger and Kendall, that it is frequently not possible to obtain an appreciable precipitation of SSS1 at this point. This matter will be discussed below. At any rate, whether or not a precipitate is obtained, the A substance is present in the acid supernatant from which it is now precipitated by alcohol and purified by precipitation with copper chloride at pH 3.4 as noted. The excess copper is removed by repeated solution in 10% acetic acid and precipitation with alcohol. The yield varies from about 100 mg. to 600 mg., from 20 L of broth culture, depending on how much SSS1 is removed by precipitation with acetic acid.

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[1] Enders, J. F., *J. Exp. Med.*, 1930, **52**, 235.

[2] Wadsworth, A., and Brown, R., *J. Immunol.*, 1931, **21**, 245.

[3] Wadsworth, A., and Brown, R., *J. Immunol.*, 1933, **24**, 349.

[4] Sabin, A. B., *J. Exp. Med.*, 1931, **53**, 93.

[5] Heidelberger, N., and Kendall, M., *J. Exp. Med.*, 1930, **52**, 477.

The A substance prepared as outlined above is an extremely hygroscopic, white amorphous powder, soluble in the range pH 1-9. It contains no sulfur or phosphorus. As recorded in Table I, its elementary analysis, amino-nitrogen content (van Slyke) and specific rotation are practically identical with the SSS1 as prepared by Heidelberger, Goebel and Avery.[6]

TABLE I *

Substance	C	H	N**	amino-N (van Slyke)	ash	αD
A	42.4	6.5	5.2	2.8	3.4	290
S	43.3	5.7	5.0	2.7	—	299

* analyses calculated to ash-free basis.
** Pregl Dumas method.

Enders,[1] whose observations have been confirmed by Wadsworth and Brown,[2, 3] found that the immunological activity of the A substance is rapidly destroyed by heating on the alkaline side of neutrality. We have observed that on boiling in N/100 NaOH, the activity of the A substance is completely destroyed in 10 minutes. The resulting solution can be precipitated at pH 3.4 with acetic acid, reacts to titre with Type I antisera, but no longer with SSS1 absorbed antiserum. This destruction of A takes place slowly, even in the cold, if solutions are kept at pH 7 or above and allowed to stand for any length of time. For this reason care must be taken during the preparation to maintain an acid reaction. The precipitin titres of A and SSS1 with a Type I rabbit anti-pneumococcus serum, unabsorbed and absorbed with SSS1 and with A, are given in Table II.

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[6] Heidelberger, N., Goebel and Avery, O. T., *J. Exp. Med.*, 1925, **42**, 727.

TABLE II

Substance	Unabsorbed Serum	SSS1 Absorbed Serum	A Absorbed Serum
A	1:4,000,000	1:4,000,000	no reaction
SSSI	1:4,000,000	no reaction	" "

It is clear that the A substance of Enders and the SSS1 are closely related. It would seem possible that the A substance more closely approximates the Type I pneumococcus haptene as present in the living cell, and that the SSS1 carbohydrate precipitated at its iso-electric point, pH 3.4, is an hydrolytic product of the A carbohydrate. In the procedure of Heidelberger, Goebel and Avery[5] for the preparation of SSS1, there can be little doubt that the A substance is destroyed at the stage where it is precipitated with $Ba(OH)_2$. In the short method for preparing SSS1, (Heidelberger and Kendall), much A is always present, carried down by the SSS1 when the latter is precipitated with acetic acid.

The question as to the true nature of the pneumococcus carbohydrates is an important one, if they are to be used in evaluating the strength of antisera. Since in many cases unabsorbed rabbit Type I antisera contain no precipitins for SSS1 whatsoever, yet precipitate heavily with A, the SSS1 is obviously of no value in estimating the strength of rabbit antisera.

The theoretical and practical implications of these observations are evident, but cannot be properly discussed until studies now in progress have been completed.

6985 C

Action of Digitalis on the Kidney.*

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It has never been clearly established whether or not digitalis has a diuretic action on the kidney aside from the secondary diuresis that occurs as a result of improvement in the circulation in congestive heart failure. A number of workers failed to obtain any direct diuretic action. Schwartz[1] was unable to produce diuresis in normal individuals with digitalis; Cushny[2] and Cohn[3] failed to ob-

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* Aided by the Frederick K. Babson Fund of the Michael Reese Hospital.

tain any increase in urinary flow in cardiac patients without edema, and Miller[4] found that digitalis had no effect on renal edema. Cushny and Lambi[5] concluded from their studies that the members of the digitalis group produced no diuresis in normal animals. However, Phillips and Bradford,[6] Pfaff[7] and Gremels[8] claimed to have obtained a diuretic action in animals with small doses of digitalis. Recently Bartram[9] has also reported that digitan has a direct diuretic action, following its injection into one renal artery.

The discrepancy in previous results led us to reinvestigate the subject. In anesthetized animals, conditions vary from time to time sufficiently to alter the urinary flow, so that results ascribed to the action of an injected drug might be coincidental. It seemed to us that the direct action of a drug on the kidney might be more readily estimated by injecting the drug directly into one renal artery and determining the urine flow of that kidney for a short period of time. Any indirect action or any spontaneous change could be eliminated by using the urine flow of the opposite kidney as a control for comparison.

Fourteen medium-sized male dogs were used. No food was given the animals for 12 hours preceding the operation, but water was urged. The animals were lightly anesthetized with nembutal intravenously (25 mg. per kilo) and this was supplemented when necessary during the course of the experiment with further injections of 5 mg. per kilo. The animal was kept on an electrically heated operating table, and all undue exposure avoided. Five hundred cc. of warm normal saline solution were injected into the jugular vein at the onset of the experiment to assure proper diuresis. The ureters were catherized individually about their middle third, through a mid-line abdominal incision. Ureteral pyelography catheters, No. 5 were inserted and held in place by silk thread. The lower ends of the ureters were tied off and the ends of the catheters brought out of the abdomen through stab wounds in the groin. The left renal artery was then isolated as it enters the superior posterior

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[1] Schwartz. (Quoted by Sollmann, T., A Manual of Pharmacology. W. B. Saunders & Co., Philadelphia. 4th edition, 1932.)

[2] Cushny, A. R., *Am. J. Med. Sci.*, 1911, **141**, 826.

[3] Cohn, A. E., *J. Pharm. Exp. Therap.*, 1915, **6**, 606.

[4] Miller, J. L., *Trans. Assn. Am. Phys.*, 1912, **27**, 137.

[5] Cushny, A. R., and Lambi, C. G., *J. Physiol.*, 1921, **55**, 267.

[6] Phillips, C. D. F., and Bradford, J. R., *J. Physiol.*, 1887, **8**, 117.

[7] Pfaff, Franz, *Arch. f. Exp. Path. and Pharmacol.*, 1898, **32**, 1.

[8] Gremels, H., *Arch. f. exp. Path. u. Pharmakol.*, 1928, **61**, 130.

[9] Bartram, E. A., *J. Clin. Invest.*, 1932, **11**, 1197.

pole of the kidney. An identifying loose ligature of silk thread was placed about it. The abdomen was then closed with hemostats and covered. (The abdominal approach is no more difficult than the more commonly employed approach from the back and less operative trauma is incurred.)

The urine from each catheter was collected and measured for a control period of 30 minutes. The abdomen was then reopened, the left renal artery picked up and the digitalis (or other) solution slowly injected into it by a syringe with a fine, short needle. If the puncture wound in the artery bled, the bleeding was controlled by a small gauze pack left *in situ*. The abdomen was closed again with hemostats and the urine flow from each ureter again collected for 30 minutes and measured. In several animals, a second control and post-injection period were run using a different strength solution of digitalis or another drug in the latter period. In some experiments no intermediate control period was run.

The position of the ureter, the absence of obstruction in the catheters and the absence of leak around them were verified in each animal antemortem and postmortem. In addition, the injected renal artery was examined for thrombosis in its lumen or wall. In none of the experiments reported were these complications present, except that occasionally intramural hemorrhage was found, which encroached slightly on the lumen.

The results are summarized in Table I. The relative urinary flow of the left (injected) kidney as compared with the right (control) kidney is given in the fifth column in percentage; and the change in this ratio following the injection of the drug is given in percentage in the sixth column. The variability in the relative and absolute urinary flow during the control periods is very marked and emphasizes the need of comparing the urinary flow of the 2 kidneys before making deductions concerning the direct action of drugs.

Using this comparison, it was found that digalen and digitalone in large doses, viz., ½ cc. of the drug (equivalent to ½ cat unit) inhibited the urine flow of the injected kidney, viz., Exp. 1, 2, 3, 6, 9, 10, 12, and 13. Ouabain (Exp. 12) had a much less striking effect even though its strength was 3 times greater (½ cc. = 1½ cat units). Some of the inhibition in the case of digalen might have been due to the alcohol used as a vehicle since it was found that alcohol in the amount and concentration used in the digalen solution caused a similar decrease in 2 experiments (Exp. 7 and 8). Even in more dilute solutions digitalone (viz., made up in a dilution of 1 to 100, equivalent to 1/200 cat unit) exhibited a direct inhibiting action on the urine flow (Exp. 10, 11, 13, and 14).

As controls, normal saline, caffeine, and salyrgan were injected in the same way. It was found that 0.5 cc. of normal saline when injected into the left renal artery had no effect (Exp. 8) or else a

TABLE I

Exp. No.	Drug injected	Urine flow in each period (30 min.) right kidney cc	left kidney cc	Ratio of urine flow on two sides L/R. %	Effect of drug on relative urine flow (change in L/R ratio.) %*	Wt. of dog Kg.
1	control	4.9	5.5	110		15
	½ cc Digalen**	3.9	2.3	60	–55	
2	control	2.2	1.8	81		7
	4/10 cc Digalen	12.0	0.9	7	–90	
3	control	12.0	6.6	55		
	½ cc caffein sod. benz.	8.4	10.0	120	+120	17
	1 cc Digalen	10.8	6.1	56	–45	
	(also 200 cc of isotonic sodium chloride intravenously)					
4	control	16.5	6.8	41		9
	1 cc Salyrgan	40.0	0.0	0	–100	
5	control	10.0	17.0	170		17
	½ cc Salyrgan	4.0	16.0	400	+135	
	½ cc Digalen	1.2	0.0	0	–100	
6	control	9.0	13.2	136		20
	2/10 cc Digalen	10.8	9.9	91	–30	
	3/10 cc Digalen	8.4	3.8	45	–50	
7	control	29.9	16.2	54		11
	½ cc alcohol (8%)	46.7	12.7	27	–50	
8	control	30.3	34.5	113		19
	½ cc isotonic NaCl	22.9	22.3	97	–15	
	½ cc alcohol (8%)	35.2	25.3	72	–25	
9	control	20.2	30.4	150		17
	½ cc Digitalone	41.4	13.4	35	–75	
10	control	9.5	21.0	221		21
	½ cc of 1:100 Digitalone	4.5	4.8	106	–50	
	½ cc Digitalone	2.9	0.0	0	–100	
11	control	6.3	33.3	530		20
	½ cc isotonic NaCl	4.5	45.0	1000	+90	
	½ cc of 1:100 Digitalone	3.5	28.1	800	–20	
12	control	29.0	33.1	114		
	½ cc Ouabain	13.8	9.9	71	–40	19
	300 cc of isotonic saline injected intravenously at this time					
	control	50.0	61.4	122		
	½ cc Ouabain	36.4	41.1	112	–10	
13	control	7.6	7.3	96		
	½ cc of 1:100 Digitalone	8.8	6.1	69	–30	22
	control	8.8	15.2	173		
	½ cc Digitalone	4.3	0.5	12	–90	
14	control	36.4	23.7	66		14
	4/10 cc of 1:100 Digitalone	32.5	2.6	8	–90	

*0 to ± 15% = no change; ± 20 to ± 35% = slight change; ±40 to ±60% = moderate change; greater than ±60% = marked change.

** 1 cc of Digalen (Hoffmann & La Roche, Inc.) and of Digitalone (Parke, Davis & Co.) = 1 cat unit.

1 cc Ouabain = 0.25 mg. = 2.5 cat units; 1 cc Caffein sodium benzoate = 0.25 gm. caffein.

1:100 Digitalone = a 1 to 100 dilution of the digitalone with isotonic saline.

stimulating action (Exp. 13). Similarly, caffeine (Exp. 3) and salyrgan (Exp. 5) had a stimulating action, although the latter when used in larger quantities (Exp. 4) actually inhibited the urine flow of the injected kidney.

Our results show that while caffeine and salyrgan have an immediate direct diuretic action, digitalis in the forms used never did. Usually digitalis had an immediate and direct inhibitory effect on urine flow. Bartram's data[9] for the first 30 minutes after injection of digitalis (his Exp. 71, 72, 73, and 74) if analyzed as we have done, shows a similar inhibition. The constant saline infusion and the partial recovery from the anesthetic which he used probably explain the progressive bilateral diuresis he observed.

In our experiments small amounts of digitalis were used, about .025 to .065 cc. of digitalone, digalen and ouabain per kilo; the toxic dose according to the figures given by Cohn and Stewart[10] would be about 1.0 cc. per kilo for the former 2, and 0.5 cc. for the last. The amounts used were made small so that the drug available to the injected kidney would be minimized. If each kidney is assumed to weigh 50 gm., then the maximum amount of drug taken up by the injected kidney would have been 10 cc. per kilo. When the undiluted drug was used this would be equivalent to 10 times the toxic dose, but when the 1/100 dilution was used this would be equivalent to 1/10 the toxic dose.

Summary. The immediate effects of digitalis preparations, digalen, digitalone and ouabain, on urine secretion were studied in anesthetized dogs by comparing the urine flow of the kidney into whose renal artery the drug was injected with the urine flow of the opposite kidney. In no instance did the digitalis preparation produce a diuretic action, in fact in most cases an inhibition of urinary secretion was noted.

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[10] **Cohn, A. E., and Stewart, H. J., *J. Clin. Invest.*, 1928, 6, 79.**

6986 C

Excretion of Inorganic Phosphate by the Aglomerular Kidney.

E. K. MARSHALL, JR., AND ALLAN L. GRAFFLIN.

From Johns Hopkins and Harvard Universities and Mt. Desert Island Biological Laboratory.

The urine of the winter toadfish, *Opsanus tau* (aglomerular kidney), contains only the faintest trace of inorganic phosphate when the fish are kept in an aquarium in the laboratory. The injection of large amounts of either monobasic or dibasic sodium phosphate intramuscularly or intravenously raises the plasma inorganic phosphate to high levels, but does not cause the excretion of any inorganic phosphate in the urine. The excretion of inorganic phosphate has not resulted from various procedures (feeding, injection of glucose, parathyroid extract, insulin). The injection of sodium glycerophosphate results in a marked rise in the inorganic phosphate of the plasma, but in no excretion of inorganic phosphate by the kidney. The above findings are interesting in view of the fact that the goosefish (another aglomerular marine teleost) secretes urine which may contain large amounts of inorganic phosphate.[1] Since we have been unable to obtain freshly caught or summer toadfish, further investigation of phosphate excretion has been carried out upon the goosefish *(Lophius piscatorius)*.

Specimens of urine obtained from the bladders of 10 freshly caught summer goosefish contained from 0.7 to 45.0 millimols of phosphate* per liter. The plasma phosphate in the same fish varied from 4.2 to 7.7 millimols per liter. The high concentrations of phosphate observed initially in the urine of some of these fish decreased markedly when the fish were kept in live cars at the laboratory.

The injection of inorganic phosphate into a goosefish does not increase the phosphate excretion. The following protocol is typical of many experiments.

Goosefish 5. 2.6 kilos.
9:54—11:56 Urine, 10 cc. containing 3.0 mM phosphate per liter (excretion 0.015 mM per hour).
11:58 Plasma phosphate, 7.7 mM per liter.
12:00 Inject intramuscularly 17 mM disodium phosphate (40 cc. of 6%).
1:52 Plasma phosphate, 19.2 mM per liter.

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[1] Marshall and Grafflin, *Johns Hopkins Hosp. Bull.*, 1928, **43**, 205.

* Whenever the word phosphate is used, inorganic phosphate is meant.

3:00 Plasma phosphate, 20.3 mM.
4:00 Plasma phosphate, 20.0 mM.
4:00 Urine, 16 cc. containing 4.0 mM phosphate per liter (excretion 0.016 mM per hour).

The result of the injection of sodium glycerophosphate is illustrated by the following experiment:

Goosefish 10. 10.0 kilos.
9:20—10:30 Urine 5.0 cc. containing 5.7 mM phosphate per liter (excretion 0.024 mM per hour).
10:30 Injection of 20.0 gm. sodium glycerophosphate (104 mM) intramuscularly
1:05 Urine 20.0 cc. containing 7.4 mM phosphate per liter (excretion 0.057 mM per hour)
2:07 Urine 5.5 cc. containing 8.2 mM phosphate per liter (excretion 0.043 mM per hour)
3:45 Urine 6.5 cc. containing 9.0 mM phosphate per liter (excretion 0.035 mM per hour)
4:45 Urine 3.5 cc. containing 8.7 mM phosphate per liter (excretion 0.030 mM per hour)
5:20 Urine 0.4 cc. containing 8.8 mM phosphate per liter (excretion 0.006 mM per hour)
5:21 Plasma phosphate, 10.7 mM per liter.

The above results prove that injected inorganic phosphate is not secreted by the aglomerular kidney and suggest that the inorganic phosphate in the urine of these fish is formed in the kidney from some precursor (which does not appear to be glycerophosphate). It is reasonable to assume that in a glomerular kidney, injected inorganic phosphate should be excreted only by glomerular filtration. If sufficient phosphate is injected to minimize the error due to reabsorption, the phosphate excretion should approximately measure glomerular filtration. This hypothesis has been tested in frogs by comparing the excretion of injected phosphate with that of xylose, which has been shown by Shannon, Jolliffe and Smith[2] to be a measure of glomerular filtration.

Xylose and dibasic sodium phosphate were injected into the lymph sac of frogs (*Rana catesbiana*), and urine and blood samples

TABLE I

Frog No.	Xylose mg % Plasma	Xylose mg % Urine	U/P Ratio	Phosphate mM per liter Plasma	Phosphate mM per liter Urine	U/P Ratio
3	162	472	2.92	7.3	20.0	2.74
5	292	876	3.00	8.5	22.4	2.64
9	194	330	1.72	9.3	21.5	2.31
	194	564	2.91	9.1	30.5	3.35
10	222	538	2.43	9.1	25.3	2.78

[2] Shannon, Jolliffe and Smith, *Am. J. Physiol.*, 1932, **100**, 301.

taken at appropriate intervals. Table I would appear to indicate that injected inorganic phosphate approximately measures glomerular filtration under these conditions.

Summary. Injected inorganic phosphate is not secreted by the aglomerular kidney but the urine of the aglomerular fish may contain large amounts of inorganic phosphate. This suggests that the inorganic phosphate in the urine of these fish is formed in the kidney from some precursor. From the above results it would appear that injected inorganic phosphate should be excreted only by glomerular filtration in the glomerular kidney. In support of this hypothesis it has been found that the clearances of inorganic phosphate and xylose agree fairly closely in frogs.

6987 P

Further Observations on Specific Inhibition of Bacteriophage Action.

PHILIP LEVINE AND ARTHUR W. FRISCH. (Introduced by Paul F. Clark.)
From the Laboratories of the Department of Pathology and Bacteriology, University of Wisconsin.

Recently we described[1] specific inhibition of bacteriophage by bacterial extracts *(B. dysenteriae Shiga* and *B. paratyphosus B).* To test further the specificity of the inhibition, experiments were made with phages and extracts of organisms within the salmonella group, namely, *B. paratyphosus B* and *B. suipestifer*. Both phages were derived from chicken stool filtrates and were specific in their reactions on the 2 cultures.

The extracts were dissolved in saline with the aid of weak alkali and heat, neutralized, diluted with saline to make a 1-200 solution, and passed through Seitz filters. Suitable dilutions of the 2 phages in beef extract broth were prepared and mixed with equal quantities of the filtered bacillary extracts. Two tests were made with these mixtures; one after incubation overnight at 37°* and another following an additional interval of 12 hours at the same temperature. One-half cc. was removed from each one of the tubes, diluted with 4.5 cc. of beef extract broth and the homologous test organism was added.

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[1] Levine, P., and Frisch, A. W., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 993.

* In other experiments it was demonstrated that the union of bacteriophage and bacterial extract progresses more rapidly at 37° than at icebox temperature.

The course of lysis in both tests showed that the action of anti-*B. suipestifer* phage was inhibited by its homologous extract, but only slightly by an extract of *B. paratyphosus B.;* on the other hand, the extract of *B. suipestifer* but weakly influenced the action of anti *B. paratyphosus B.* phage which was inhibited to a considerable degree by the *B. paratyphosus B.* extract. The effects were pronounced in both tests but the results were somewhat better in the mixtures incubated for the longer period.

Furthermore, the course of lysis of the anti-*B. suipestifer* phage was normal when placed in contact with extracts of *B. stanley, B. aertryck,* and *B. dysenteriae* (Shiga).

Other experiments were made to determine the relationship between phage titre and inhibition by extracts of various organisms acted on by a related phage. For this purpose, anti-*B. paratyphosus B.* phage was employed together with extracts of several smooth *phosus B., B. aertryck, B. stanley, B. tidy,* and also a smooth resistant strain of *B. paratyphosus B.* derived from the overgrowth following phage action on this culture. This organism, still resistant to phage 10 months after its isolation although transplanted numerous times, could not be differentiated serologically, morphologically, or biochemically from its parent culture.[3, 4] Crude saline extracts of these strains were prepared and tested for their capacity to inhibit the action of anti-*B. paratyphosus B.* phage. The tests demonstrated that all these extracts specifically retarded the action of the phage, but to a variable degree. The most intense inhibition was exhibited by an extract of *B. stanley* and the poorest by that of *B. aertryck.* Apparently the order of activity of the extracts does not run parallel to the titre of the phage on the living bacillus since the Stanley strain was acted on least (10^{-1}) but its extract gave the most intense inhibition. Further support for this contention was found in the behavior of the resistant strain, an extract of which gave an inhibition more distinct than that of *B. aertryck* although the latter organism was lysed to a titre of 10^{-5}.

The failure to establish a relationship between phage inhibition by extracts and the phage titre against the living bacillus, suggested other ideas, namely, the phage absorbing capacity of the whole bacillus, either living or dead, the precipitin titre of the bacillary

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[2] White, P. B., Great Britain Med. Res. Council, Special Rep. Series, No. 103. 1926; *System of Bact.*, 1929, **4**, 115.

[3] Burnet, F. M., *J. Path. and Bact.*, 1929, **32**, 349.

[4] Bronfenbrenner, J., Muckenfuss, R. S., and Korb, C., *J. Exp. Med.*, 1926, **44**, 607.

extracts, or both. Investigations along these lines are in progress; it may be stated in a preliminary way, however, that, on the whole, there was a rough parallelism with the precipitin titre; secondly, in our hands, the heat-killed resistant *B. paratyphosus B.* organism specifically absorbed the anti-*B. paratyphosus B.* phage.[5, 6, 7]

To gather more information on the specificity of the reaction, the bacillary preparations employed in the experiment mentioned above and also that of *B. dysenteriae* (Shiga) were tested against anti-*B. dysenteriae* (Shiga) phage and inhibition was obtained only in the case of the homologous substance.

The specific behavior of extracts of *B. suipestifer* and the *B. paratyphosus B.* group of organisms toward their homologous phages indicates that the method may lend itself to a classification of the Salmonella group of organisms in terms of bacteriophage action perhaps in a manner similar to the serum reactions with the whole bacillus or extracts thereof.[8] Should other instances be found where extracts of resistant organisms specifically inhibit the phage derived against the parent strain, this technique and perhaps also absorption by dead bacilli would offer the advantage of revealing relationships to be expected from a serological point of view, but not demonstrable by direct phage action.

The demonstration of phage inhibition by solutions of bacillary extracts supplies a reaction for chemical studies on bacillary antigens in terms of bacteriophage.

6988 P

Attempts to Transmit Poliomyelitis to Mice, Rats, Guinea Pigs and Rabbits.*

JOHN A. KOLMER AND ANNA M. RULE.

From the Research Institute of Cutaneous Diseases, Philadelphia, Pa.

The successful transmission of poliomyelitis to the commoner and cheaper laboratory animals would be of inestimable assistance

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[5] Applemans, R., *Comp. rend. soc. biol.*, 1922, **86**, 508.

[6] Gohs, W., and Jacobson, I., *Z. f. Immunitats.*, 1927, **49**, 412.

[7] Flu, P. C., *Centr. f. Bakt.*, I Orig., 1923, **90**, 374.

[8] Furth, J., and Landsteiner, K., *J. Exp. Med.*, 1929, **49**, 727.

* Investigation aided by a grant from the Dr. Daniel J. McCarthy Fund for Research in Neurology of Temple University.

to investigations upon infection, immunity and specific therapy of this disease. Since Flexner and Lewis[1] recorded the failure of dinitrophenol (hereinafter called αDNP) increases the rate of goats, pigs, sheep, rats, mice, dogs and cats, various other investigators have attempted transmission to the lower animals but with uniform failure except in the case of monkeys and apes.

Now that it is known that syphilis may be transmitted to mice, rats and guinea pigs and that *Spirochaeta pallida* may survive in the lymph glands, brain (especially of mice) and other organs for long periods of time without any clinical signs or symptoms of the disease, we have sought to transmit the virus of poliomyelitis to mice, rats, guinea pigs and rabbits by injecting them intracerebrally with 0.1 to 0.2 cc. of 10% emulsions of monkey poliomyelitic spinal cord.

Four animals of each group were inoculated under ether anesthesia and after an interval of 3 weeks, during which time the 16 animals were carefully observed for any possible signs of infection, all were chloroformed and the brains removed. A 10% emulsion of the anterior lobes of the cerebrums of the 4 mice was prepared and injected intracerebrally into 4 fresh mice, the emulsions being prepared from the sites of the former injection. Similar emulsions were prepared of the inoculated sites of the brains of the 4 rats and inoculated into 4 fresh rats; similar emulsions were prepared of the guinea pig and rabbit brains and likewise inoculated into 4 fresh guinea pigs and rabbits respectively. After 3 weeks of observation this procedure was repeated a third time and finally a fourth time before the experiments were terminated. It was our hope that some virus may persist and gradually acquire pathogenicity but in every instance none of the large number of mice, rats, guinea pigs or rabbits developed the slightest clinical evidences of poliomyelitic infection nor could we find any histological evidences of infection in sections of the spinal cords.

In one instance we inoculated a monkey intracerebrally with 0.2 cc. of a 10% emulsion of rabbit brain removed 3 weeks after inoculation with monkey poliomyelitic cord (Rockefeller Institute), but this monkey did not develop poliomyelitis, suggesting that the virus failed to survive in the brain of the rabbit.

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[1] **Flexner, S., and Lewis, P. A.,** ***J. Exp. Med.,*** **1910, 12, 227.**

6989 P

A Note on the Chemotherapy of Experimental Poliomyelitis of Monkeys.*

JOHN A. KOLMER AND ANNA M. RULE.

From the Research Institute of Cutaneous Medicine of Philadelphia.

Probably because only monkeys are known to be susceptible to the virus of poliomyelitis among the lower animals, little thought and attention have been given to chemotherapeutic investigations in this disease on account of the expense involved. Furthermore the results of medicinal treatment of acute anterior poliomyelitis in human beings have yielded no interesting or encouraging "leads" or suggestions for chemotherapeutic investigations and individual case reports have so far failed to receive confirmation. Young, Hill and Scott[1] have recorded alleged benefit in the treatment of 2 cases of poliomyelitis with mercurochrome by intravenous injection, but this compound given to other cases under the personal observation of one of us did not result in any curative activity.

Having on hand a number of *Macaccus rhesus* monkeys which had been used for experiments on vaccination against poliomyelitis[2] but which developed the disease following intracerebral inoculation with the virus as a test for any possible acquired immunity, we have thought it worth while to institute treatment with a variety of chemical compounds as soon as definite signs and symptoms of poliomyelitis developed which terminated the experiments on vaccination. Under these conditions the experimental disease was allowed to produce pronounced paralysis before the drugs were given by intravenous injection but we thought it worth while to use the animals in these therapeutic tests with the hope that some encouraging results for further work in this field may be secured.

The compounds selected, the doses per kilogram of weight and the number given each animal are shown in the accompanying table. All compounds were administered by intravenous injection every 3 days for 5 to 8 injections. Each compound was given to 1 or 2 animals. As previously stated all of the monkeys showed unmistakable paralysis developing 6 to 9 days after intracerebral inoculation with virus before treatment began.

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* Aided by a grant from the Dr. Daniel J. McCarthy Fund for Research in Neurology of Temple University School of Medicine.

[1] Young, Hill and Scott, *Arch. Surg.*, 1925, **80**, 813.

[2] Kolmer, J. A., and Rule, A. M., *J. Immunol.*, in press.

TABLE I

Ineffectiveness of following compounds in experimental poliomyelitis of monkeys.

Compounds	No. Treated	Dose per Kilo*	No. Doses	Results
Hexamethylamine	2	.025 gm.	8	died
Metaphen	2	.0005 ”	8	”
Mercurochrome	1	.005 ”	6	”
Pregl's solution of iodine	2	.5 cc.	8	”
Sodium and gold thiosulphate	1	.0003 gm.	7	killed
Sodium ricinoleate	2	.005 ”	5	”
Neoarsphenamin	1	.02 ”	8	died
Neutral acriflavin	1	.010 ”	6	”
Tryparsamide	2	.03 ”	6	”
Bismuth Potass. Tartrate	1	.002 ”	5	killed

*By intravenous injection every 3 days; treatment begun upon first signs of poliomyelitis following intracerebral inoculation with virus 6 to 9 days previously.

In every instance paralysis progressed under treatment; 7 succumbed to the disease while the remaining 3 were chloroformed when it was apparent that treatment was ineffective.

6990 C

Concerning the Absorption of Unsplit Protein Through the Lacteals.*

BENJAMIN M. BANKS.† (Introduced by W. C. Alvarez.)

From the Mayo Foundation, Rochester, Minnesota.

It is commonly assumed that allergic responses to ingested foods are due to the passage of unchanged protein from the bowel into the blood. Alvarez, who had seen a few cases in which unfavorable reactions to food appeared to be worse when patients ate much fat with the offending protein, suggested to me that an unguarded pathway might be through the lacteals and thoracic duct into the blood stream. In this way the protein would escape possible change in the intestinal wall and in the liver. Somewhat in favor of this view is the reported observation that food reactions are less likely to occur if the patient first takes a dose of hydrocarbon oil; theoretically, it might block the lacteals.

A number of investigations, inspired by the contributions of Uhlenhuth,[1] and of Hamberger and Sperk,[2] attempted to identify unchanged protein in the blood of animals by means of the precipitin reaction, but unfortunately the results were conflicting. The studies of Hektoen, Kanai, and Dragstedt[3] may be mentioned in this regard. The difficulties in detecting foreign protein in the blood by precipitin methods are considerable, not only because the material is so greatly diluted, but, as has been frequently shown, because it leaves the blood stream so rapidly. The work of Walzer[4] and of Smyth and Stallings,[5] in which the newer principle of passive transfer in human beings was utilized, indicated that a detectable amount of certain proteins frequently enters the blood in unaltered form by way of the intestine. More recently, the experiments of Nedzel and Arnold[6] on the influence of egg-white on the absorption of bacteria from the intestinal tract, and the studies of McDaniels[7] on the effect of egg white in oral vaccination of mice against pneumococci, have given new impetus to studies in this field.

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* Work done under the direction of Drs. H. E. Essex and W. C. Alvarez, Division of Experimental Medicine, The Mayo Clinic.

† Residence now in Boston, Massachusetts.

[1] Ascoli, M., and Vigano, L., *Z. f. physiol. Chem.*, 1903, **39**, 283.

A survey of the literature shows that others in the past have searched for foreign protein in the lymph of the thoracic duct with the help of the precipitin reaction. Ascoli and Vigano[8] in studying such absorption noted the unexpected presence in rabbit serum of precipitin bodies for fasting dog lymph. This has been confirmed by others so that positive results in such experiments may be open to question. Recently, Ishikawa[9] reported his inability to detect unaltered egg albumin in the thoracic duct lymph of dogs after oral administration. He could find it only when he operated on the animals and injected the protein directly into a loop of intestine. Matsui and Iida are quoted by Ishikawa as having obtained results similar to his, but their papers are not accessible to me.

Since the results of precipitin tests can now be controlled by newer immunologic procedures, the problems of absorption of unsplit protein through the lacteals was subjected to further investigation. The present communication briefly summarizes the data obtained in this study.

1. *Preparation of anti-egg-albumin rabbit serum.* Eight rab-

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[2] Coca, A. F., and Grove, E. F., *J. Immunol.*, 1925, **10**, 445.

[3] Hamberger, F., and Sperk, B., *Wien. klin. Wchnschr.*, 1904, **17**, 641.

[4] Hektoen, Ludwig, Kanai, P. H., and Dragstedt, L. R., *J. Am. Med. Assn.*, 1925, **84**, 114.

[5] Ishikawa, Issaku, *Jap. J. Med. Sc.*, IV. Pharmacology, 1928, **2**, 205.

[6] McDaniels, H. E., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 587.

[7] Nedzel, A. J., and Arnold, Lloyd, PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 358.

[8] Smyth, F. S., and Stallings, M., *J. Allergy*, 1931, **3**, 16.

[9] Tomcsik, Joseph, and Kurotchkin, T. J., *J. Exp. Med.*, 1928, **47**, 379.

bits were given a series of alternating intravenous and subcutaneous injections of egg albumin at intervals of 4 days, starting with 1.5 cc. of a 1% solution in saline per kilo of body weight, and doubling the amount with each injection. Those rabbits the serum of which attained a precipitin titer of 1:80,000 to 1:100,000 were bled under sterile conditions and the serum was stored in the ice-box.

2. *Collection of lymph, technic of feeding.* Under ether anesthesia, the left internal jugular vein of fasting dogs, weighing 12 to 13 kg. was found with aseptic precautions, and all venous connections were ligated so that only the thoracic duct drained into it. A glass cannula was fastened in the vein and connected with a length of rubber tubing which was brought out to the surface through a stab wound. The original incision was closed in the usual manner. From 1½ to 2 hours after the animals had recovered from the operation samples of lymph were collected. With the animal resting, further specimens of lymph were obtained at intervals. Heparin was used as an anticoagulant. Later, food was given by stomach tube.

3. *Precipitin reactions.* The material to be tested (lymph from the fasting animal, physiologic saline controls, known and unknown concentrations of egg albumin in saline solution or lymph) was layered over anti-egg-albumin rabbit serum in minute test tubes 3 mm. in diameter and 30 mm. long. After standing for one hour at room temperature, a record of the condition at the surface of contact was made. It was determined by control experiments that the heparin did not interfere with the precipitin reaction.

4. *Technic of Tomcsik and Kurotchkin.*[10] As outlined by these investigators, guinea pigs weighing 250 to 280 gm. were passively immunized by the intraperitoneal injection of from 2 to 4 cc. of anti-egg-albumin rabbit serum. Twenty-four hours later, the intravenous administration of 1 cc. of 1 to 1,000 or 1 to 2,000 egg albumin solution (1 mg. to 0.5 mg.) to such animals invariably resulted in typical anaphylactic shock and death.

5. *Technic of Prausnitz and Kustner (atopic reagins).* By the method of Coca and Grove,[11] 0.1 cc. of anti-egg-albumin rabbit serum was injected intracutaneously in a series of rabbits, and after an interval of half an hour samples of material to be tested were similarly injected at the same sites. The animals were observed for 2 hours, and again at the end of 24 hours, for evidence of local reaction.

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[10] Uhlenhuth, *Deutsch. med. Wchnschr.*, 1900, **26**, 734.

[11] Walzer, Matthew, *J. Immunol.*, 1927, **14**, 143.

Unfortunately precipitin reactions between the immune rabbit serum and control samples of lymph from fasting dogs were common, and in only 4 experiments were such phenomena absent. Unaltered egg albumin in the thoracic duct lymph was not demonstrated. The simultaneous feeding of fat in one experiment did not cause a detectable amount of unsplit protein to appear in the lymph. Ascoli and Vigano attached significance to the fact that if lymph of fasting dogs gave faint precipitates with immune rabbit serum, the feeding of egg albumin produced heavier precipitation. This was true in our experience, but the feeding of 500 cc. of whole sheep blood caused similar effects, showing that the effect is not specific.

By means of anaphylactic reactions[9] absorption of unaltered protein through the lacteals could not be shown. All guinea pigs used in these tests were subsequently given intravenous injections of 1 cc. of 1:1,000 egg albumin in physiologic saline solution or in control lymph of fasting animals approximately one hour after the first intravenous injection, with death in every instance by anaphylactic shock.

Attempts to detect unaltered egg albumin by utilizing atopic reagins (method of Coca and Cole) were entirely unsuccessful.

A possible objection to the work may be that raw egg white is perhaps the least desirable protein to be used in such experiments, since it is often so poorly absorbed in the bowel of the dog.

Conclusion. In the dog, absorption of unaltered egg-albumin from the intestinal tract by way of the lacteals and thoracic duct could not be demonstrated.

6991 P

A Test for the Demonstration of Estrin in the Blood of Women.*

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From the Department of Obstetrics and Gynecology, Stanford University School of Medicine.

It was previously shown[1] that a mucification of the vaginal mucosa of adult spayed mice may readily be induced by the injection of blood serum from women in the premenstruum or in early preg-

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* Supported in part by a grant from the Rockefeller Fluid Research Fund of Stanford University School of Medicine.

nancy. At that time it was generally believed that this change in the test animal was brought about by a specific corpus luteum hormone, but the more recent studies of Robson,[2] Robson and Wiesner,[3] and Meyer and Allen[4] indicate that it is an effect of small doses of estrin.

The induction of mucification of the vaginal mucosa of adult spayed mice may thus be employed as a test for the presence of estrin in the blood of women with various clinical conditions. The procedure at present in use consists of injecting a total of 4.5 cc. of clear blood serum into mice which have been castrated for from 5 to 8 days. The animals are injected subcutaneously 3 times daily with 0.5 cc. and are sacrificed in 72 hours. The vagina is dissected free, fixed in formalin, and mounted in paraffin. Sections are made at different levels and stained with hematoxylin-eosin.

Including the 25 cases previously reported,[1] tested by a slightly different technique, 57 observations have been made on the blood of women at various phases of the menstrual cycle. Of 10 women examined during the early stage of proliferation (days 4 to 7 of a 30-day cycle), 2 gave a positive result and 8 negative; of 15 in the late stage of proliferation (days 8 to 14) 9 were positive and 6 negative; of 18 in the early stage of secretion (days 15 to 21) 15 were positive and 3 negative; of 7 in the late stage of secretion (days 21 to 28) 6 were positive and one negative; of 3 examined within 48 hours of the appearance of the menses one was positive and 2 negative; and of 4 during menstruation one was positive and 3 negative.

These results compare favorably with those found with the method of Frank and Goldberger. This test, in addition, offers a distinct advantage in that the small amount of serum employed enables several mice to be injected with each specimen and no extensive chemical extraction of the blood is required.

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[1] Fluhmann, C. F., *Am. J. Physiol.*, 1930, **95**, 422.

[2] Robson, J. M., *J. Physiol.*, 1931, **71**; *Proc. Physiol. Soc.*, iii.

[3] Robson, J. M., and Wiesner, B. P., *Quart. J. Exp. Physiol.*, 1931, **21**, 217.

[4] Meyer, R. K., and Allen, W. M., *Science*, 1932, **75**, 111.

6992 P

Action of 1-2-4 Dinitrophenol on Yeast Respiration and Fermentation.*

J. FIELD 2ND, A. W. MARTIN AND S. M. FIELD.

From the Department of Physiology, Stanford University.

Cutting and Tainter,[1] Magne, Mayer and Plantefol,[2] Hall, Field, Sahyun, Cutting and Tainter[3] and others have shown that 1-2-4 dinitrophenol (hereinafter called αDNP) increases the rate of oxygen consumption of animals by direct action on the tissue cells. Similar action on some plants has been demonstrated by Plantefol.[4] However, Genevois and Saric,[5] during the course of our experiments, reported that αDNP failed to increase yeast respiration, in fact that a concentration of 100 mg. per liter decreased respiration and the percentage of viable cells by 90%. These results will agree with ours as to respiration if the pH was below 6.0.

We have studied the action of αDNP on the respiratory and fermentative processes of pure cultures of *Saccharomyces cerevisiae*, race F, over a considerable concentration range and at various acidities, using Warburg[6] respirometers at 25°C. The respiration studies were carried out in an atmosphere of pure oxygen, those on fermentation (method of Negelein[7]) in 95% N_2 and 5% CO_2.

αDNP was used in the form of the sodium salt, which in no case shifted the pH from the level set by the buffers (glass electrode pH determinations were kindly made by Dr. F. DeEds). Nor did the pH level change during the course of the run.

αDNP is an acid with a pK of 4.02 at 25°C. The ions are yellow, the undissociated acid is colorless.[8] Our experiments indicate

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* Supported in part by a grant from the Rockefeller Fluid Research Fund of the Stanford University School of Medicine.

[1] Cutting, W. C., and Tainter, M. L., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1268.

[2] Magne, H., Mayer, A., and Plantefol, L., *Ann. Physiol. Physicochim. Biol.*, 1931, **7**, 269.

[3] Hall, V. E., Field, J. 2nd, Sahyun, M., Cutting, W. C., and Tainter, M. L., *Am. J. Physiol.*, in press.

[4] Plantefol, L., *Ann. Physiol. Physicochim. Biol.*, 1932, **8**, 127.

[5] Genevois, L., and Saric, R., *Compt. rend. Soc. de biol.*, 1932, **111**, 181.

[6] Warburg, O., *Uber den Stoffwechsel der Tumoren*, Berlin, 1926.

[7] Negelein, E., *Biochem. Z.*, 1925, **158**, 121.

[8] Michaelis, L., and Rona, P., *Praktikum der Physikalischen Chemie*, Berlin, 1930.

that only the undissociated form is biologically active, as the following data show.

RESPIRATION

pH	Total conc. DNP in mg. per l.	Conc. undissociated form in mg. per l.	Effect
5.2	400	25.08	Inhibition
5.9	10	0.13	Stimulation
	50	0.65	" (optimal)
	100	1.30	Inhibition
	400	5.20	"
6.8	Below 50	Below 0.08	No effect
	100	0.16	Stimulation
	400	0.64	" "
	800	1.28	Inhibition
8.03	400	Negligible	No effect

In all cases proper controls were run.

Similar findings were obtained in the studies on fermentation. Within certain limits the action is reversible. At pH 5.9, a total concentration of 400 mg. per liter inhibits strongly. If after one hour under these conditions enough alkali is added from the sidearm of the Warburg vessel to bring to pH 6.8, thus decreasing the concentration of the undissociated form, strong stimulation results. This holds for both respiration and fermentation.

The effect is independent of the presence of glucose, and the onset occurs within 5 minutes of addition of the αDNP.

Depression of respiration at pH 6.8 is not accompanied by death of cells (staining with Methylene-blue after Fink and Kühles[9] and plate counts).

The stimulation range of the undissociated form for yeast is near the same order of magnitude as that found for animals.[2, 10]

The contradictory results of other investigators may be ascribed to lack of appreciation of the activity of the undissociated form, and consequent use of unsuitable hydrogen ion concentrations. It may be that the biological inactivity of the ionic αDNP is associated with permeability relations. Experiments are now in progress on this and other points.

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[9] Fink, H., and Kühles, R., *Z. physiol. Chem.*, 1933, **218**, 65.

[10] Cutting, W. C., Mehrtens, H. G., and Tainter, M. L., *J. Am. Med. Assn.*, 1933, **101**, 193.

6993 P

Vitamin E and Pituitary Hormone. I. Failure of Vitamin E Preparations to Induce Precocious Sexual Development.

F. A. DIAKOV AND J. KRIZENECKY.

From the Department of Breeding-Biology, Zootechnical Research Institute, Brno, Czechoslovakia.

(Publ. No. 215.)

Verzár and his collaborators[1, 2] observed that the extracts of wheat-germs containing vitamin E, when applied intraperitoneally or subcutaneously, bring about the same biological reaction as the hormone from the anterior lobe of pituitary gland, *i. e.*, oestrus and hypertrophy of the uterus (based on precocious activation of ovaries).

The purpose of our experiments was to reproduce Verzár's results and thus to use this phenomenon as a convenient means of standardization of vitamin E preparations,* and further to compare the activity of vitamin E with that of pituitary hormone and prolan A. Three active vitamin E preparations were used, the potency of which was proved by the Evans-Burr method.

For testing vitamin E preparations (I and II) 3 groups (6 in each) of infantile female rats (30-35 days) were used. The first and second groups received daily intraperitoneally, for 14 days, 0.3 cc. of vitamin E preparations Nos. I and II, respectively. The third group serving as control received the same amount of olive oil. Experimental and control animals gained in body weight and seemed healthy. No external signs of stimulation of sex development such as hypertrophy and hyperemia of labiae vaginalis and opening of vagina were observed. On dissection also we found no signs of maturity, *i. e.,* neither ripening of follicles nor hypertrophy and hyperemia of uterus and tubes.

Similar tests were made with vitamin E preparations No. IIIa and IIIb. Three animals of the first group, and 2 of the second died after receiving 5 doses (0.3 cc.) while the other animals survived as many as 12 doses each. On the fifth day the animals were examined, killed and dissected. The experimental animals lost much weight, the skin of abdominal regions was wet, and the ab-

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[1] Szarka, A., *Pflüger's Arch.*, 1930, **223**, 657.

[2] Verzár, F., *Pflüger's Arch.*, 1931, **227**, 499.

* The preparations were made for us (details not published by the Company) by the Pharmaceutical Department of the Chemical Manufacturing Co., Kolin, Czechoslovakia.

domen was distended showing that, when given intraperitoneally, these preparations were toxic. No signs of hastened maturity of sexual organs were observed, but rather a tendency of the uterus and tubes to atrophy.

Parallel with vitamin E experiments prolan A (from pregnancy urine) and hormone prepared from anterior lobe of hypophysis were tested. The preparations were administered subcutaneously as well as *per os*. In all cases enhanced maturity was observed, i. e., ripening of follicles, opening of vagina and great hyperemia and hypertrophy of labiae vaginalis, uterus and tubes. In case of subcutaneous administration the reaction followed within 80-100 hours and the prolan preparations were active in very small doses, while in cases of peroral administration the reaction followed in 160-180 hours and was not so noticeable and larger doses were required.

We were unable to repeat Verzár's findings, and conclude that vitamin E does not induce sexual maturity as does anterior pituitary hormone and prolan A; this method therefore can not be used for standardization of vitamin E preparations.

6994 P

Vitamin E and Pituitary Hormone. II. Failure of Ant. Pituitary Hormone and Prolan A to Substitute Completely Vitamin E.

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(Publ. No. 216.)

In disagreement with reports of Verzár and his collaborators, results obtained by us[1] on young rats indicate that vitamin E has a biological action different from that of gonadotropic anterior pituitary hormone and prolan A. In a different type of test we have used mature female rats which were raised on vitamin E-free diet and have tested the capacity of gonadotropic pituitary hormone and prolan A to induce fertility in them as does vitamin E.

These rats, raised on vitamin E-free diet and all more than 3 months old, were divided (May 30) into 2 groups (I and II); both groups being left on vitamin E-free diet. Group I received twice a week 0.5 cc. of wheat-germ extracts as a source of vitamin

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[1] PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 58.

E. Since the rats showed no changes this dose was increased (August 3) to 6 x 0.5 cc. weekly. Even this quantity seemed too small and the dose was again increased (August 19) to 6 x 1.0 cc. weekly for 3 animals (Nos. 122, 123 and 135). A pregnant female (No. 130) was left on the previous dose (6 x 0.5 cc.) of vitamin E extract and this animal on August 27 gave birth to 6 healthy young. Nos. 122, 124 and 135 also gave healthy litters of average size, but even at this dose No. 123 resorbed her fetuses.

The rats of group II (on vitamin E-free diet and serving as control for group I) did not produce any young from May 30 to August 9. During this period these rats increased in weight and were fertilized but the fetuses died and were resorbed. From August 9 three animals received (1.0 cc. daily) pituitary gland hormone and the other 3 the same quantity of prolan A (from urine). Both preparations had very high gonadotropic activity as shown by tests on infantile female rats. On August 13 administration of both preparations was temporarily discontinued; from August 20 to September 12 the animals again received 1.0 cc. daily of pituitary hormone and prolan A. All females had become pregnant and increased in weight but none of them had given birth to young. On November 12 this group was transferred to normal Sherman diet; within 10 to 15 days all of them became pregnant, and all gave birth to healthy young, showing that the fertility of the female rats was not destroyed but only suspended by lack of vitamin E.

These experiments show that neither pituitary hormone nor prolan A can completely replace vitamin E; they merely aid the first phase of embryonic development. Evidently the biological action of vitamin E is not identical with that of pituitary hormone and prolan A. The hormone preparations may favor ovulation, implantation of the fertilized egg, and early embryonic development; but vitamin E is necessary for further development of the fetus and the completion of gestation.

Our thanks are due to Dr. J. Spinka, Director of the Chemical Manufacturing Co., Kolin, Czechoslovakia, whose financial support enabled us to complete these experiments.

6995 P

B. Tuberculosis in Psychotic Patients (Loewenstein's Method).

NICHOLAS KOPELOFF AND E. LOEWENSTEIN.

From the Departments of Bacteriology of the Psychiatric Institute, N. Y., and the Staatliches Serotherapeutisches Institut, Vienna.

A cooperative experiment was undertaken concerned with the direct cultivation of *B. tuberculosis* by the Loewenstein method from the blood of psychotic male patients at the Psychiatric Institute and Hospital.

Seven cc. of blood were introduced aseptically into a tube containing 3 cc. of a 10% solution of sodium citrate, sealed and sent by parcel post to Vienna. Bloods from male physicians and male attendants were sent as controls. Triplicate specimens of blood were taken from each subject at the same time. All tubes were numbered serially in New York. Professor Loewenstein did not know that the specimens were in triplicate. Neither did he know the diagnoses of any patients, nor that control subjects were included.

A careful physical examination of *all* patients and subjects by Dr. M. M. Harris, Research Associate in Internal Medicine, failed to reveal any clinical signs of active tuberculosis. This was further confirmed by radiographic examination. Dr. L. E. Hinsie, Research Associate in Psychiatry, not only vouched for the psychiatric diagnoses but took all of the blood samples. We are privileged to acknowledge our indebtedness to them.

Professor Loewenstein reported all of his findings before being informed of the diagnoses, etc. The results briefly summarized are as follows:

	No. Examined	No. + Macro. & Microscopic	No. + Microscopic Only
Dementia Praecox	34	15	5
Psychoneuroses	4	2	1
Manic-Depressive	2	1	
Inv. Melancholia	2	2	1
Total	42	20	7
Controls	12	0	0

Upon visiting Professor Loewenstein in his laboratory this summer after the findings were recorded, we found 13 cultures reported as macroscopically positive which had not yet been discarded. These were brought back to New York and guinea pigs were inoculated. (One culture was dried out.) All of the guinea pigs came down with tuberculosis within 5 weeks after inoculation. All showed strongly positive tuberculin tests, local lesions at the site of injection, gross evidence of tuberculosis in the inguinal glands, spleen and liver, and stained smears revealed typical acid-fast bacilli. Cultures from these organs yielded macroscopic growth in all cases. Histological examinations proved positive.

This investigation is now being repeated and extended but so far has definitely established that *B. tuberculosis* could be recovered from the blood of approximately half of 42 male psychotic patients examined by the Loewenstein method in the absence of any clinical signs of tuberculosis, while the blood of 12 controls remained negative.

The interest and assistance of Dr. C. O. Cheney, Director, was invaluable during this investigation as was that of Lenore M. Kopeloff and John L. Etchells in our laboratory.

6996 P

Somatic Myogenic Action in Embryos of Fundulus Heteroclitus.

G. E. COGHILL.

From the Wistar Institute of Anatomy and Biology, Philadelphia.

Five phases in the development of motility in this species can be recognized. (A) Localized spontaneous contractions within the trunk (part attached to the yolk-sac) only; tail-bud, immotile. (B) All movements spontaneous; integrated contractions throughout the trunk, but slow in execution and relaxation; localized contractions only in the proximal part of the tail; distal part, immotile. (C) The general integrated contractions are quick; involving about the proximal half of the tail with the trunk; localized contractions only in the most distal myotomes of the region involved in the general integrated contractions, or just behind it, the more distal myotomes being immotile. This phase is marked by the beginning of tactile sensitivity. (D) The general contractions involve the entire trunk and tail; localized spontaneous contraction only at the tip of the tail and in the pectoral fin. (E) Localized movement of the tail has ceased, and the pectoral fin, like the caudal fin, becomes integrated with the total action pattern, without capability of local reflexes.

The skin of the embryo and yolk-sac being highly impervious to curare, the drug was injected into the yolk-sac, or the latter was cut open while immersed in the solution in order to distinguish myogenic from neurogenic action. In embryos of phase A, under the action of curare, contraction of the myotomes occurred as much as 15 minutes after the circulation of the blood had ceased. This is accepted as conclusive evidence that all muscular contraction of this phase is purely myogenic. In embryos of phase B, injected with curare, spontaneous movements are localized at different levels within the trunk and originally motile part of the tail: integration of the system as a whole is lost. Compression of the fourth ventricle, in some cases, excites movements in all the myotomes that were originally contractile, but in others it excites movement only in the originally motile myotomes of the tail. The contraction of more posterior myotomes in response to compression of the cerebrospinal fluid while the anterior myotomes are passive is accepted as conclusive evidence that these movements are myogenic, for if they were excited through the nervous system the parts nearest the center of compression would also respond. The posterior myotomes which act in this manner are those which normally undergo spontaneous localized contraction. All the myotomes that are normally contractile are capable of myogenic action, but those in the base of the tail are purely myogenic, the more anterior being normally neurogenic. Embryos of phase C, treated with curare, become insensitive to tactile stimulation, but may yet respond to compression of the fourth ventricle. In some there occurs general spontaneous contractions in all the myotomes that are normally contractile, but in most of them spontaneous contraction occurs only in the tail. Infrequently compression of the fourth ventricle excites general flexures, but mostly only contractions in the tail and particularly in its distal half. According to the criteria used in connection with phase B, all of the normally contractile muscles are capable of myogenic action in some specimens but not in all, the normally neurogenic system has extended a considerable distance into the tail, the purely myogenic system has moved farther tailward, and the nonmotile system involves only the tip of the tail. The myotomes that normally undergo localized spontaneous contractions are purely myogenic, although under normal conditions the more rostral of the myogenic series may be stimulated, probably by mechanical stress, to act with the neurogenic system. Fishes of phase D, within a few minutes after injection, become insensitive to touch, but the entire tail in some specimens moves spontaneously or in response to compression of the fourth ventricle, while in others only the tip of the tail moves under these conditions. Late in this phase the pectoral fins move spontaneously for 9 minutes or more after the fish has become insensitive to touch. In phase D, therefore, the myotomes of the trunk are purely neurogenic in action, while the movements of the caudal and pectoral fins are purely myogenic, and most of the tail, although potentially myogenic in function, is normally neurogenic.

The axial somatic muscles and those of the pectoral fin are first purely myogenic and finally purely neurogenic in action, and between these pure states is an intermediate one in which the contractions are potentially myogenic but normally neurogenic.

6997

Effect of Parathormone on Bone Phosphatase Activity in vitro.

HARRY BAKWIN AND OSCAR BODANSKY.

From the Children's Medical Service and Department of Pathology, Bellevue Hospital, and the Department of Pediatrics, New York University.

The relationship between hormonal and enzymic activity is of considerable biological interest. Kay[1] noted high plasma phosphatase values in generalized *ostitis fibrosa.* Page[2] reported a decrease in the bone phosphatase in rats after parathormone injections. Bodansky and Jaffe[3] obtained increases in plasma phosphatase 8 hours after injection of single large doses in the dog and decreases in the guinea-pig after 1 and 2 days.

Heymann[4] reported a direct effect of parathormone *in vitro*. He observed decreases of 50 to 100% in bone phosphatase activity when 0.1 units of parathormone were added per cc. of hydrolysing mixture. The effect on kidney and intestinal phosphatase was much less marked. He conducted his experiments at a pH of 7.7 using a 50% concentration of a phosphatase preparation which had been extracted from the tissue with glycine buffer. Sodium glycerophosphate and hexosephosphate were employed as substrates. The parathormone was added in a glycine solution.

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[1] Kay, H. D., *J. Biol. Chem.*, 1930, **89**, 249.

[2] Bodansky, A., and Jaffe, H. L., *J. Biol. Chem.*, 1931, **92**, XVI.

[3] Page, I. H., *Biochem. Z.*, 1930, **223**, 222.

[4] Heymann, W., *Biochem. Z.*, 1930, **227**, 1.

We have studied the effect of parathormone* on the phosphatase activity of aqueous extracts of rat and cattle bone. The sodium diethylbarbiturate buffer of Michaelis was used. This buffer in 0.5% concentration is without effect on phosphatase activity and hence is to be preferred to glycine which, in the concentrations used for buffering, retards phosphatase activity about 30%.[5] The technique of tissue phosphatase activity estimation was similar to that used in previous studies.[5] 0.0127 M sodium β glycerophosphate (Eastman Kodak) was used as the substrate. The reciprocal of the time in minutes necessary to liberate 0.05 mg. of inorganic phosphate as P per cc. of hydrolyzing mixture was used as the measure of enzyme activity (Q).

For each activity determination, a series of 4 hydrolyses was conducted in the neighborhood of the optimal pH. The amount of phosphate liberated was estimated at varying time intervals. The reaction velocity (Q) was determined by graphic interpolation from the time-change curve in the hydrolysis tube showing optimal activity.

Two different rat bone extracts and one cattle bone extract were used. The enzyme concentration, parathormone concentration, and temperature were all varied.

TABLE I
Phosphatase Activity (Q)

Units parathormone per cc. hydrolysing mixture	Rat Bone (Preparation R B D) 12.5 % 24° c.			Rat Bone (Preparation R B E) 24° c.		Cattle Bone (Preparation C B E) 30°c
				25%	50%	50%
0.0	.0077	.0081	.0081	.0068	.0149	.0471
0.1	.0079					.0463
0.5	.0076			.0074		.0464
2.5	.0081	.0077	.0084		.0162	

The results are shown in the table. Parathormone has no effect *in vitro* upon the β glycerophosphatase activity of bone extracts of the rat or cow.

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* We wish to express our thanks to Eli Lilly and Company for supplying the parathormone.

[5] Bakwin, H., and Bodansky, O., *J. Biol. Chem.*, 1933, **101**, 641.

6998 P

Mass Ligation of the Pancreas Near the Head in Diabetes Mellitus.

HARRY KOSTER, WILLIAM S. COLLENS AND BENJAMIN S. GESHWIN.

From the Crown Heights Hospital, Brooklyn, N. Y.

The surgical approach to the treatment of diabetes mellitus has taken 4 routes in the human. Goldjanitski[1] and others ligated the parotid duct but with little success. Dubious results have been obtained by adrenal denervation (Ciminata[2]) and by liver denervation (Depisch *et al*[3]). Mansfeld's[4] studies in dogs established the pancreas as the fourth method of approach. He ligated the tail of the pancreas in dogs and obtained results which indicated an increase in sugar tolerance. DeTakats and Wilder[5] modified Mansfeld's technique and instead of ligating the tail of the pancreas, they isolated it by section with cautery and wrapped it in omentum. This operation was performed in one case of juvenile diabetes. Subsequently DeTakats[6] employed the original Mansfeld technique in 2 other cases of juvenile diabetes but used a piece of fascia for the ligature. In none of his cases did he get any noteworthy improvement.

Four years ago, we studied the effects of mass ligation of the pancreas about one inch from the head of the gland in dogs. Histological examination of the pancreas 6 to 8 weeks after this operation revealed a shrinkage of the caudal portion to about one-tenth of the original size, due to extensive fibrosis in the entire acinar tissue of that portion. The only remaining parenchyma consisted of markedly hypertrophied islet tissue.

We felt that patients with diabetes mellitus might be improved if the ligation were nearer the head of the pancreas. The consequent acinar atrophy would be of greater extent and the concomitant changes in the islet tissue of greater magnitude. Three patients suffering from severe diabetes mellitus were therefore treated in this manner.

For several weeks before operation, the patients' carbohydrate tolerance was studied. They were operated under spinal analgesia. Two pieces of cotton binding tape, one-quarter inch width, were passed around the pancreas immediately caudal to the point of entrance of the superior pancreatico-duodenal artery into the gland and they were successively tied as tightly as possible. The wound was then closed without drainage.

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[1] Goldjanitski, quoted by Depisch *et al.*

[2] Ciminata, A., *Arch. di pat. e clin. med.*, 1929, **8**, 79.

[3] Depisch, F., Hasenohl, R., and Schonkauer, L., *Wen. klin. Worchschr.*, 1930, **43**, 321.

[4] Mansfield, G., *Klin. Wochschr.*, 1924, **3**, 2378.

[5] DeTakats, G., and Wilder, R. M., *J. Am. Med. Assn.*, 1929, **93**, 606.

[6] DeTakats, G., *Surg. Gynec. and Obst.*, 1931, **53**, 45.

Our criteria of carbohydrate tolerance was the glucose-insulin quotient previously discussed (Collens[7]). All studies were made under identical conditions of diet and insulin intake.

No deleterious effects of the operation could be noted. In the first case there was no obvious improvement in the glucose-insulin quotient during the first year. In the second and third years after the operation the glucose-insulin quotient rose slightly. Three and a half years after the operation, the patient still has a marked hyperglycemia and glycosuria. The glucose-insulin quotient shows little improvement since it was 2.7 before the operation and is now 4.7. Clinically the patient showed improvement very early and today appears in a very good state of nutrition.

The second and third cases likewise showed very little change in their tolerance to carbohydrates, although only 3 months have elapsed since operation.

These results tend to indicate that if there is a hyperplasia of the Islands of Langerhans in man as in the dog following ligation near the head of the pancreas, this does not improve the patient with diabetes mellitus. This operation should result in the production of a larger amount of insulin than if only the tail of the pancreas were ligated. But hyperplasia of a large part of the islet tissue did not produce a commensurate increase in the production of insulin, or the increased amount of insulin is inactivated by a factor responsible for the disease. This suggests confirmation of the thesis that diabetes mellitus may be due to some extra-pancreatic disturbance.

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[7] Collens, W. S., and Grayzel, H., *Am. J. Dis. Child.*, 1929, **38**, 275.

6999 P

Lesions in the Pyramidal Tracts in Cats.*

CLYDE MARSHALL. (Introduced by H. S. Burr.)

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The present experiments attempt to produce lesions confined to the cortico-spinal (pyramidal) tract in cats. Theoretically, this might be done in the pyramids of the medulla, and both pyramids should be cut to remove all cortico-spinal innervation from even one side of the body. This operation, with survival long enough for Marchi studies, has been described only by Starlinger[1] (dogs) and by Schäfer[2] (monkeys). Lesions of one pyramid only, or of the pyramidal decussation have been reported by a few others.

In the present series, 12 bilateral and 4 unilateral lesions were made in the pyramids in cats. Survival was permitted for varying periods to 24 days. Nembutal and sodium amytal were used as anesthetics. The pyramids were cut through an incision in the ventral aspect of the neck. The findings reported begin with the second day and unless otherwise specified refer to the animals with bilateral lesions.

Posture. The assumption of temporary grossly abnormal postures was observed initially in 5 of the 12 bilaterally operated animals and disappeared in these after a few days. Milder manifestations of defective postural control, such as slipping of the feet on a smooth floor, undue twisting of the pivoting leg on a turn, or an over extension of the toes with a backward position of a limb, were present in practically all animals initially. They almost or entirely disappeared.

Of the more constant early postural abnormalities the most common was a tendency to stand with the hind legs abducted and sometimes also inwardly rotated. Less frequently the forefeet appeared unduly flattened on the floor. Many of the animals assumed at times a peculiar half sitting position, resting the whole of the hind feet from toes to ankle on the floor with the rump some distance above it. They often settled into this position with a peculiar slowness and moved slowly from it into other postures. These phe-

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* Aided by a grant from the Rockefeller Foundation Fluid Research Fund of Yale University School of Medicine.

[1] Starlinger, J., *Neurol. Centralbl.*, 1895, **14**, 390; *Jahrb. f. Psychiat. u. Neurol.*, 1897, **15**, 1.

[2] Schäfer, E. A., *Quart. J. Exp. Physiol.*, 1910, **3**, 355.

nomena usually disappeared with time. The animals rarely spontaneously put their weight on the dorsum of a foot, but if a foot was placed in the turned over position by the examiner, the normal correction movements were absent for from 4 to 21 days.

Motion. Gross difficulty in walking, marked staggering, or swaying appeared in only 4 of the series and in these it lasted no more than a day. A rather constant abnormality of gait was an apparent stiffness in the hind limbs, which were brought forward with less than the normal play in the distal joints. The forelegs usually showed no defects in walking, but in a few cases a certain heaviness or plodding was noticed. A striking feature was the frequent occurrence of a marked reduction in general activity. This was manifested in the early days by little spontaneous walking and later by a slow or unhurried motion. Running, galloping, or general friskiness were rarely observed in the bilateral animals even at the end of 3 weeks, although by this time little or no disturbance in the gait itself was usually apparent. Walking along the rungs of a horizontal ladder, or along a narrow track often brought out defects when walking on the floor appeared to be normal. Jumping down from a height or walking on the hind legs only, showed certain other deficiencies. But even these functions were capable of marked improvement.

Placing and Hopping Reactions. The flexion movements obtained by stroking the hairs of the feet (Munk's Berührungs reflex) or by bringing the dorsum of the feet lightly against a table edge (Stehbereitschaft of Rademaker) were absent initially but eventually returned. The visual stehbereitschaft was temporarily impaired. The hopping reactions showed an initial delay, *i. e.*, the leg had to be bent far from the vertical before the hop would begin, and the resulting movement was in many cases excessively forceful and heavy. Marked or total recovery eventually took place.

Tone. The resistance of the limb to passive flexion (extensor tonus) was almost always increased initially, particularly with certain postures of the animal. This could usually be readily demonstrated by suspending the animal ventrally in a sling where the increased resistance was observable in all limbs, or by holding it with one hand under the belly where it could be seen in the hind legs. When lying on its back in a trough this resistance was usually less marked than in the other positions described. In general, ventral stimuli appeared to increase, dorsal stimuli to inhibit it. Along with the increased resistance to flexion went a diminution of the flexor activity normally incited by passive manipulation of the limbs, a function most readily measured by resistance to passive extension. Both these phenomena gradually passed away, with a certain tendency for the diminished flexor activity to persist for a time after the resistance to passive flexion had disappeared.

General. After unilateral operation, the contralateral limbs were chiefly but not exclusively involved. The homolateral hind limb was damaged to a lesser extent and the homolateral forelimb hardly at all. In general, also, the contralateral limbs were somewhat less disordered after unilateral, as compared with the bilateral operations. Marchi degeneration studies are in progress.

7000 C

Relative Utilization of Calcium from Calcium Carbonate and Calcium Gluconate by Chickens.

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From the Departments of Agricultural and Biological Chemistry and of Poultry Husbandry, the Pennsylvania State College.

Numerous investigations have indicated that the chicken may use a number of forms of calcium for its metabolic needs. Workers at the Kentucky Experiment Station[1] studied calcium carbonate, calcium lactate, calcium sulphate, tri-calcium phosphate and calcium chloride as sources of calcium for chickens and found that calcium carbonate was the most effective judged by the degree to which it was utilized in the production of eggs, its influence on the weight of egg contents and shells, and quantity of the salt consumed. Calcium sulphate was not so effective as the carbonate as shown by a smaller egg production and lower weight of shells and egg contents. Calcium lactate was readily utilized but the quantity consumed was variable and small as compared to the carbonate and sulphate. Only small quantities of calcium chloride were consumed and the precipitated tri-calcium phosphate was not a satisfactory source of calcium for egg production as compared to calcium carbonate. The above mentioned salts were fed as supplements to a wheat, yellow corn and skim milk ration.

Bethke[2] and his associates state that no difference was found in the availability of calcium in the carbonate, sulfate, lactate and phosphate salts when fed to chicks on a basis of equal calcium intake on a minimum requirement basis.

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[1] Buckner, G. D., Martin, J. H., and Peter, A. M., *J. Agr. Res.*, 1928, **36**, 263.

[2] Bethke, R. M., Kennard, D. C., and Kick, C. H., *Poultry Science*, 1929, **9**, 45.

As calcium gluconate is a readily soluble salt, it was thought desirable to study it as a source of calcium as compared to calcium carbonate, since the carbonate is the commonly used source of supplemental calcium for poultry. The following studies include both growing chicks and laying hens.

Two groups each consisting of 81 day-old Barred Rock chicks were placed in battery brooders and given rations that differed only as to the source of calcium. Group I received calcium carbonate as its supplemental source of calcium, while Group II received calcium gluconate.* These salts were incorporated with the ration which was fed in all-mash form and kept before the birds at all times. Table I gives the make-up of the starting and growing ration as well as the ration used for the laying birds in the second part of the experiment.

TABLE I

	Starting and growing ration		Laying ration	
	Ration I	Ration II	Ration I	Ration II
Ground yellow corn	34.5	35.2	27.9	29.46
" wheat	15	15	15	15
" oats	20	20	20	20
Alfalfa meal	10	10	10	10
Meat meal	5	5	5	5
Fish "	5	5	5	5
Dried Milk	5	5	5	5
Calcium carbonate	1		2.2	
" gluconate		4.32		9.5
Sodium phosphate			.8	.8
Commercial sucrose	4		8.9	
Cod liver oil	.5	.5	.2	.2
	100	100	100	100
% calcium	1.38	1.40	1.86	1.88
% phosphorus	0.657	0.662	0.848	0.851

The sugar was added to the calcium carbonate rations to compensate for the gluconic acid carried by the gluconate rations.

The males were discarded at the end of 6 weeks, and at 8 weeks of age, 8 pullets were removed from each group, the left tibia was dissected from each and bone ash determinations made on the oven dry fat-free tibiae. Group I exhibited an average bone ash of 49.8%, Group II a bone ash value of 48.9%. Since vitamin D had been supplied in ample quantity it was thought advisable to study the bone ash of birds that had been fed the same rations as were fed to Groups I and II but to restrict the cod liver oil to 0.10% of the total ration. Previous observations had shown that when this level of cod liver oil was administered, many birds became rachitic at an early age. Two groups of 15 chicks each were used in this study and when bone ash determinations were made at 8 weeks, the birds receiving calcium carbonate had an average bone ash of 37.6 and the ones receiving calcium gluconate had an average bone ash of 39.2 No further studies were made with these birds.

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* The calcium gluconate used in the study was obtained through the courtesy of Röhm and Haas Co., Inc., of Bristol, Pa.

When the birds that were started on the original experiment were 22 weeks old, they were taken from the batteries and 12 birds from each group were placed in individual compartments of laying cages. At this time the average weights of the birds in Groups I and II were 1,450 gm. and 1,535 gm., respectively. The results with growing chicks are in agreement with those of Hart[3] and associates, who compared calcium carbonate, calcium sulfate, bone meal, rock phosphate, dicalcium phosphate and calcium gluconate as sources of calcium for the growing chick in the presence of ample quantities of vitamin D. They state that: "These findings indicate clearly that it cannot be assumed that one form of calcium is better than another for animal feeding because it is more soluble in water. The rate of solution of calcium salts in the animal intestine is sufficient, even in case of the more insoluble compounds to make all forms equally effective to the animal provided adequate vitamin D is present."

The rations fed to the birds after they were placed in the laying cages differed only slightly from the rations they received during the growing period. It was necessary, however, to add more calcium to the ration to take care of the increased demand for calcium for the production of egg shells. The phosphorus content of the rations was also raised, by the addition of sodium phosphate. It was felt that in this study any differences that might result from the use of the 2 salts would be more apparent if vitamin D were supplied in limited amounts, consequently 0.2% of cod liver oil was supplied in the ration which was fed as all mash and kept before the birds at all times. This amount of cod liver oil furnished vitamin D in quantity less than that required for good egg production from birds denied access to sunlight. The laying birds in this experiment were housed in a basement room and any sunlight reaching them was filtered through window glass. The composition of the ration used for egg production is given in Table I.

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[3] Hart, E. B., and Deobald, H. J., Wisconsin Bulletin 421.

After egg production started the following data were collected: number and weight of eggs produced, weight of shell, weight of shell ash, and in some cases the calcium level of egg contents was determined. Limited studies were also made on the hatchability of eggs and livability of the resultant chicks. The birds continued on the study for 12 months after egg production started and egg shell studies were made except for 2 months during which time the eggs were used for hatchability studies. The determination of calcium in egg contents was not started until after the birds had been in production for 3 months and was discontinued while the eggs were being used in hatchability studies.

Eggs were collected daily and were weighed the following day, at which time the shells were removed, washed free from adhering contents, dried to constant weight and ashed. For the most part the shells from a number of eggs were pooled for the ash determinations. Table II gives the shell and shell ash determinations by months, the percentages being based on the weights of the original eggs. Table II also includes results of calcium determination on egg contents.

TABLE II

Month	Shell Group I %	Shell Group II %	Shell Ash Group I %	Shell Ash Group II %	Ca in egg contents Group I %	Ca in egg contents Group II %
1	7.9	8.8	4.1	4.7		
2	8.3	8.5	4.3	4.5		
3	7.6	8.5	3.9	4.4	.051	.053
4	8.0	8.9	4.1	4.5	.050	.053
5	8.1	9.0	4.3	4.7	.051	.053
6	8.0	9.1	4.2	4.8	.050	.053
7	8.3	8.8	4.5	4.7	.050	.052
10	8.4	8.9	4.6	4.9	.050	.052
11	8.3	8.8	4.4	4.6	.050	.052
12	8.3	8.7	4.3	4.5	.051	.052
Average	8.13	8.79	4.27	4.62	.050	.053

Calcium determinations were made on pooled samples of eggs from the 2 groups and in order to obtain a representative sample, the eggs were broken and stirred for 10 minutes with an electric stirrer before samples were taken for analysis. The contents of eggs produced by Group I had an average analysis of .050% calcium and the eggs produced by Group II had an average of .053% calcium for the 8 months during which these determinations were made.

Since it was observed that the calcium level of the egg contents was somewhat higher in the group receiving calcium gluconate, it was thought desirable to determine the relative amounts of thick and thin albumen present in the eggs produced by the 2 groups of birds. This was done using the method and apparatus described by Holst and Almquist.[4]

Group I produced an average of 105 eggs per hen during the year, with an average weight of 50.1 gm. per egg, while Group II in the same time produced an average of 115 eggs with an average weight of 50.5 gm.

Seven birds out of the 12 in Group I that were started in the second part of the experiment survived and 8 birds of the 12 that were started in Group II were alive at the completion of the study. All hens were individually mated and observations made as to the fertility and hatchability of eggs produced by the 2 groups. The fertility in both groups was quite low but no difference between the groups was noted. The hatchability of fertile eggs was satisfactory for both groups. Because of the small number of birds involved, the hatchability data are not presented.

In the presence of ample vitamin D, calcium gluconate seems to function as well as calcium carbonate as a source of calcium for the growing chick. The same is true when these supplements are supplied in rations where vitamin D is limited. The birds receiving calcium gluconate grew slightly better than those receiving calcium carbonate. In rations fed to the laying hen, where vitamin D was supplied in quantity less than the optimal, calcium gluconate appears to be somewhat more efficient than calcium carbonate in the formation of egg shell, as evidenced by greater weight of egg shell and a higher percentage of shell ash. Calcium gluconate under the conditions involved in these experiments also caused a greater deposition of calcium in the inner parts of the egg. It has been generally believed that the calcium content of the interior parts of the egg is constant and not influenced by feeding or other treatment of the hens, but Hughes and associates[5] were able to raise the calcium level of the egg contents by irradiating the hens producing the eggs.

During the first few months that observations were made on the amount of thick white present, it appeared that the eggs produced on the calcium gluconate ration had a higher percentage of thick white than those produced on the calcium carbonate ration. This difference tended to lessen as the study proceeded and the average percentage of thick whites from the 2 groups was not substantially different when calculated for the entire period.

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[4] Holst, W. F., and Almquist, H. J., *Hilgardia* 6, **48**, 1931.

[5] Hughes, J. S., Payne, L. F., and Latshaw, W. L., *Poultry Science*, 1923, **3**, 151.

Due to differences in the calcium content of the 2 salts studied, it was necessary to use more than 3 times as much gluconate as carbonate to maintain an equal calcium level. The use of calcium gluconate in such amounts as were used in this study is likely to be impractical from a cost standpoint, but it would be desirable to determine whether or not the gluconate has a supplemental effect when the major part of the calcium in the ration is furnished by calcium carbonate or some other source of calcium. The level of vitamin D was decidedly lower than the optimum level and it is possible that the birds could function as well as they did under the conditions of this experiment, if lower levels of calcium were used in the presence of ample vitamin D.

The number of birds involved in the egg production studies was quite small but the differences observed in shell, shell ash and calcium level of the egg contents seem to be significant.

Summary. Calcium carbonate and calcium gluconate seem to function with equal efficiency as sources of calcium for the growing chick when equivalent amounts of calcium are supplied. With the laying hen the calcium gluconate seems to function slightly more efficiently than the carbonate, as evidenced by increased egg shell, shell ash, and an increase of calcium in the egg contents.

7001 C

The in vivo Action of Staphylococcus Bacteriophage in Presence of Staphylococcus Antitoxin.

EARL L. BURKY.

From the Wilmer Institute of Ophthalmology, Johns Hopkins University and Hospital.

In a previous report[1] it was shown, as it has been by others, that normal rabbit serum in broth inhibits the action of staphylococcus bacteriophage on staphylococci. However, when rabbit serum, immune to staphylococcus toxin was used in the place of normal serum, the bacteriophage remained active and subcultures taken from the higher concentrations of bacteriophage were sterile. This finding suggested that a combination of staphylococcus antitoxin and bacteriophage might be an efficient therapeutic agent in the treatment of staphylococcus infections. The results of such therapy, which will be reported elsewhere in detail, were discouraging. However, an effect was obtained which seems to show an *in vivo* action that parallels the previously observed *in vitro* action.

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[1] Burky, E. L., *J. Immunology*, 1933, **24**, 513.

Nineteen rabbits were injected in the right marginal ear vein with 1 cc. per kilo of a 48 hour broth culture of *Staphylococcus aureus,* strain Fs. Blood cultures were obtained from the left marginal ear vein 24 hours later. Six rabbits were then injected in the right ear vein with staphylococcus antitoxin (10 cc.) and bacteriophage (2 cc.). Six rabbits were injected with bacteriophage alone (2 cc.). The bacteriophage was active against strain Fs in dilutions of 1×10^{-9}. The remaining 7 rabbits were kept as controls. This procedure was repeated daily until the 5th day when all of the treated animals, except one treated with bacteriophage alone, were dead. A similar mortality occurred in the untreated animals.

Three of the animals treated with bacteriophage and antitoxin and 4 of the rabbits treated with bacteriophage alone were alive 72 hours after the injection of the bacteria. The blood cultures taken at this time and shown in the photograph were typical of the results

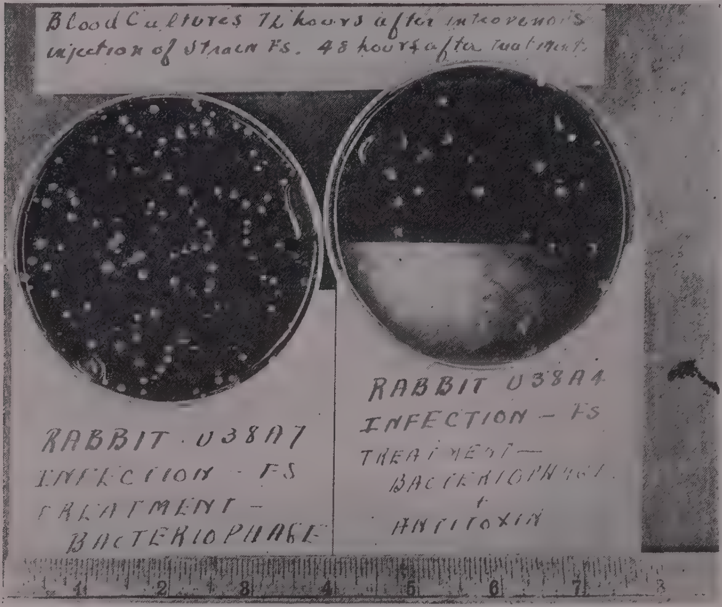

FIG. 1.

obtained. The blood cultures of the animals treated with bacteriophage alone showed no evidence of the bacteriophage. The blood cultures of those treated with antitoxin and bacteriophage showed phaged colonies.

The presence of bacteriophage in these colonies was determined by subcultures on plain agar slants and in broth tubes. The plain agar subcultures showed the characteristic ragged growth. The broth tubes were filtered after 24 hours' growth and bacteriophage was found in the filtrates by the serial dilution method previously described[1] Bacteriophage could not be found in the colonies from the rabbits treated with bacteriophage alone or with antitoxin alone. There was no change in the colonial form of the staphylococci recovered from the animals treated with antitoxin alone.

From these results it is apparent that the antitoxin has some effect on the bacteriophage that allows it to survive and act on the staphylococci in the presence of normal rabbit blood. No explanation of this phenomenon can be offered at this time.

7002 P

Structure and Function of the Yolk-sac Placenta in Mus norwegicus albinus.

JOHN W. EVERETT. (Introduced by J. S. Nicholas.)

From the Osborn Zoological Laboratory, Yale University, and the Department of Anatomy, School of Medicine, Duke University.

Although the relative functions of the yolk-sac and allantoic placentas in rodents are still uncertain, the following considerations indicate that the former has greater importance than is generally recognized. Goldmann[1] observed that the highly colloidal acid-azo dyes do not enter the chorionic villi of the rat placenta, but pass through Reichert's membrane and are stored superficially in the "yolk-sac epithelium." Brunschwig[2] has demonstrated that the yolk-sac is the route of entrance of iron salts into the 9-day blastocyst. Furthermore, the present experiments show that the neutral red accumulation in the yolk-sac epithelium exceeds that in any other embryonic tissue when an early blastocyst is placed in a solution of the dye. Also, when embryonic loci of from 11 to 13 days' gestation are perfused by way of the uterine artery with a solution of toluidin blue in saline, the dye passes into the embryo by way of the yolk-sac and the vitelline veins. When pregnant females of 16 or 18 days are injected intravenously with trypan blue, the only embryonic tissue in which dye is found 1 to 2 hours later is the portion of the yolk-sac epithelium which rests against the placental disc. The dye diffuses through the base of the placenta, rather than through the extremely thin "plates" of the chorionic villi.

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[1] Goldmann, E. E., *Beiträge z. klin. Chirurg.*, 1912, **64**, 1.

[2] Brunschwig, A. E., *Anat. Rec.*, 1927, **34**, 237.

The yolk-sac epithelium is composed of columnar cells. An ellipsoidal or spherical nucleus is located at the base of each cell until the 10-day stage and then is displaced upward by a cluster of fat droplets. Beneath the cuticular border of the cell is a granule-containing layer. Between it and the nucleus lies a region which is vacuolated in fixed material.

This vacuolation, first described by Sobotta,[3] is not a fixation artifact. In sections of material fixed in Zenker-formol or chrome-osmic mixtures a distinct wall may be seen about the structure. Osmic acid blackens the wall faintly about the more apical margins and densely along the inferior borders, especially in the frequent perinuclear diverticula. In Zenker-formol preparations stained with Altmann acid-fuchsin, the entire element, when contained within a single section, stands out definitely as a reddish sac. It can be seen in unstained pieces of epithelium mounted in hanging drop, and is conspicuous when certain supravital stains are employed.*

The selective staining of the yolk-sac by neutral red or trypan blue is due to the large amount of dye which these intracellular vesicles absorb. Nile blue sulphate, which ranges from deep blue in aqueous solution to orange-amber in neutral fat, with intermediate colors in certain lipoid mixtures, stains the vesicle a pale violet. Spherical granules of varying sizes at its surface are dense blue. In epithelium stained with toluidin blue, brilliant cresyl blue, thionin, or aged methylene blue, the dye is reduced to the leuco base in the lower regions of the cell and remains intensely colored in the supranuclear region. Here the color is not the blue of a watery solution, but reddish violet. This effect is partly due to the assumption of the violet color by the vesicle. Densely blue short rods cling to the vesicle's surface or lie within, where they are in rapid Brownian movement. Wherever these metachromatic dyes are red or violet one may assume that they are there dissolved in a substance of low dielectric constant. The osmic acid and Altmann techniques, indicate that such a substance, a lipoid, is localized in the wall of the vesicle.

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[3] Sobotta, J., *Arch. f. mikr. Anat.*, 1911, **78**, 271.

* Observations were made of hanging drop preparations with an aprochromatic oil-immersion objective and compensating oculars.

The shape of the vesicle is in constant flux, diverticula being slowly formed, retracted, or fused together. On several occasions spherical bodies have been seen to form from these diverticula. One of the bodies became highly refractile and meanwhile its color changed to the orange-amber of the fat droplet in the base of the cell.

All observations indicate that the vesicle is an extremely irregular sac, having a lipoidal wall, and filled with a fluid of low viscosity. Although the composition of the wall and the relation to the nucleus suggest that the element is the Golgi vacuome, such a conclusion seems premature until further investigations have been made.

7003 C

Pinacyanol as a Histological Stain.

FREDERICK PROESCHER.

From the Pathological Laboratory, Santa Clara County Hospital, San Jose, Calif.

Pinacyanol is a dye belonging to the cyanine group. During the last 10 years great advances have been made, chiefly by Mills[1] and his coworkers, in the preparation and elucidation of the chemical constitution and photographic properties of the cyanine dyes. There are about 37 different types of cyanine dyes known.

The cyanine dyes have the peculiar property of conferring extra sensitiveness on silver halides, which are only sensitive to the blue and violet regions of the spectrum. If the proper dyes are added to the emulsion, or if the films are bathed in the solution, they become extremely sensitive to red, yellow, orange, green and to the invisible infra-red portions of the spectrum. This sensitizing property is probably due to the following characteristic molecular grouping present in all sensitizing cyanine dyes:

Ring with tertiary nitrogen $\rangle$ $(:CH\cdot)_n$ $\langle$ Ring with quaternary nitrogen

Where $n = 0, 1, 2, 3$.

(One equivalent of an acid radical and 2 nitrogen atoms of basic function joined together by a chain of conjugated double bonds)

The cyanine dyes are strongly basic dyes, the monoacid salts are colored while the diacid salts are colorless. The chemistry of the sensitizing action is unknown. However, we do know that the dye is adsorbed by the silver halide.

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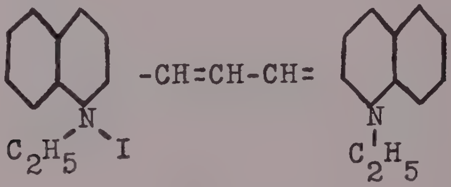
[1] Doja, M. Q., *Chem. Rev.*, 1932, **11**, 3.

The cyanine dyes* available commercially are: Orthochrome T (sensitizer for green), Pinacyanol (sensitizer for red), Kryptocyanine (sensitizer for extreme red), Dicyanine A (sensitizer for infra-red), Neocyanine (sensitizer for infra-red) and Quinoline blue.† These dyes, with the exception of quinoline blue‡ have not been used for histological purposes. The staining properties were investigated for various tissues. Pinacyanol was the most satisfactory, not only as a polychromatic stain cat exogen but also for its quick staining action. Naphtocyanol and Dicyanine A were less satisfactory but may be used for special purposes. Kryptocyanine and Orthochrome T stained diffusely without differentiation. Neocyanine had no staining effect at all.

Pinacyanol has the following formula:



It is formed by the condensation of quinaldine ethiodide on itself in the presence of formaldehyde and contains 3 methylenyl groups between the two rings. Pinacyanol iodide crystallizes from alcohol in the form of fine blue-green needles. The alcoholic solution is dichroitic; in reflected light it is blue and in transmitted light it is red. Dissolved in pyridin it becomes a pure blue with slight dichroism and on adding water, the color changes to violet-red.

For histological purposes 0.5 gm. of pinacyanol are dissolved in 100 cc. absolute ethyl or methyl alcohol. However, this solution may be further diluted with alcohol in proportion 1:3 or 1:5 according to the required staining intensity. The solution will keep indefinitely if placed in clean, glass-stoppered bottles, preferably pyrex glass, and protected from the light. The aqueous solution is not stable and deteriorates in a short time.

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* These dyes may be obtained from the Eastman Kodak Co., Rochester, N. Y.

† Quinoline blue (chinolinblau) may be obtained from Dr. Gruebler, Leipzig.

‡ Quinoline blue (Greville Williams, 1856), the oldest cyanine dye, was first used by Certes (*American Microscopic Journal* No. 3, 1883) in very dilute solutions 1:100,000 to 1:500,000 for staining infusoria. When used in these dilutions it will stain only the fat granules. Ranvier advocated a very dilute alcoholic solution of the dye for staining fresh as well as preserved objects. Vivante used the dye for staining decalcified bone; Walker for staining vegetable fat (*Int. Monatsschrift f. Anat.*, 1882, **9**); Golenkin for the detection of free or bound iodine (*Bull. Soc. Nat. Moscou*, 1899); Zimmermann for staining cellulose membranes.

Pinacyanol gives the best results with either native or fixed frozen sections. Formol or Formol-Mueller fixation gives equally good results. It may be used in paraffin or celloidin embedded material, but the brilliancy and differential staining of the various tissue elements is entirely lost. For staining frozen sections, the section is mounted on a slide and covered with a few drops of the alcoholic staining solution which is allowed to remain for from 5 to 10 seconds; the section is then floated from the slide into distilled water, washed for about 15 seconds, remounted, and embedded in C. P. glycerin.

Pinacyanol is a nuclear as well as a protoplasmic stain. The chromatin ranges in color from dark blue to cobalt violet and is very distinctly differentiated. The protoplasm is stained in various shades of purple. It further differentiates connective tissue from elastic and muscle tissue. The connective tissue is stained eosin-red, the elastic tissue a deep black-violet and the muscle tissue in different shades, ranging from blue-violet to purple. The neutrophile and eosinophile granules are not stained while the granulo-plasma of the plasma cells is stained a brilliant carmine-red, standing out prominently from all other inflammatory cells. The hemoglobin remains unstained while the neutral fat is either colorless or presents a faint bluish-violet hue. The various lipoids are stained a deep bluish-violet to deep purple. Pinacyanol is also an excellent stain for amyloid, which is almost instantaneously stained a distinct carmine-red without any further differentiation. The hemosiderin is stained a brilliant orange-yellow.

Glycerin seems to intensify the staining effect. The intensity of the color of the granulo-plasma of the plasma cells and of the elastic fibers increases considerably after the sections are mounted in glycerin. The permanence of the staining effect depends greatly on the kind of tissue and the state of preservation. Frozen sections of tissue fixed in Formol or Formol-Mueller have so far retained their color for a period of 7 months. Stained frozen sections can not be dehydrated and embedded in balsam. Alcohol decolorizes practically all of the protoplasmic substances while the chromatin remains more or less intensely stained.

Pinacyanol offers the opportunity for the most detailed cell studies, not only from a morphological but from a histo-chemical standpoint.

7004 P

Effect of Hypophysectomy upon Pregnancy and Lactation in Mice.

H. SELYE, J. B. COLLIP AND D. L. THOMSON.

From the Department of Biochemistry, McGill University, Montreal.

In the rat, hypophysectomy during lactation leads promptly to cessation of milk secretion;[1] hypophysectomy during the second half of pregnancy does not usually prevent the birth of living young nor the development of the mammary glands, in fact milk secretion begins normally at parturition but ceases within 26 hours.[2] Caesarian section shortly before term regularly induces lactation, but fails to do so if the pituitary is removed.[3] Mammary development in virgin rats under the influence of ovarian hormone secretion stimulated by the anterior-pituitary-like hormone leads to milk secretion when the luteinized ovaries are removed, but this reaction also does not occur in the absence of the pituitary.[4] These findings are in accord with the view of Riddle,[5] Turner,[6] and others that a specific hormone of the anterior pituitary is responsible for the actual initiation of lactation, and that a sudden decrease in the amount of ovarian hormones in the circulation is a stimulus for the production of this hormone (Nelson and Smelser[7]), but they also indicate that the contents of the pregnant uterus may take the place of the anterior pituitary in this reaction.

We have recently succeeded in hypophysectomizing albino mice by a parapharyngeal operation, almost identical with the modified Smith procedure used by us in rats and described elsewhere.[8] In the mouse the pituitary is situated somewhat more caudally, relative to the spheno-occipital synchondrosis; the bone is therefore drilled exactly over this synchondrosis. We have experienced no trouble from hemorrhage, no mice have been lost during the operation, and the post-operative mortality has been somewhat lower than in rats.

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[1] Collip, J. B., Selye, H., and Thomson, D. L., *Nature,* 1933, **131**, 56.

[2] Selye, H., Collip, J. B., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 589.

[3] Collip, J. B., Selye, H., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 913.

[4] Selye, H., Collip, J. B., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 588.

[5] Riddle, O., Bates, R. W., and Dykshorn, S., *Am. J. Physiol.,* 1933, **105**, 191.

[6] Gardner, W. U., and Turner, C. W., *Mo. Agr. Exp. Sta. Bull.,* 1933, **196**.

[7] Nelson, W. O., and Smelser, G. K., *Am. J. Physiol.,* 1933, **103**, 374.

[8] Collip, J. B., Selye, H., and Thomson, D. L., *Virchows Arch. path. Anat.,* 1933, **290**, 23.

Six mice, 3 months of age, were hypophysectomized on the second or fourth day of lactation. All recovered from the operation and attempted to rear their litters, but milk secretion failed and within 24 hours the stomachs of the young were empty.

Six mice, 3 months of age, were hypophysectomized during the second half of pregnancy. Abortion did not occur; as the date of impregnation was not known, it could not be ascertained whether parturition was delayed; however, it took place normally in every case, and the litters were normal in size and gross appearance. The young were born dead, or at least were dead when first observed a few hours after birth, whereas 22 out of 24 rats hypophysectomized during gestation bore living young.[2] The mice resembled the rats in that milk secretion occurred; this was shown by the escape of milk when the gland was incised, and confirmed by histological study.

These experiments in general confirm our previous work on the rat. We intend to describe the effects of hypophysectomy in the mouse in more detail elsewhere; it may be mentioned here that the corpora lutea regress rapidly after hypophysectomy, whereas in the rat they preserve their normal appearance for a considerable time.

7005 P

Susceptibility of Eels and Dogfish to Diphtheria Toxin; Toxicity of Eel Serum and Dogfish and Skate Plasma.

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From the Division of Laboratories and Research, New York State Department of Health, New York.

The action of the bacterial toxins depends upon tissue susceptibility; but the mechanism by which they act and the conditions in the tissues determining their action are quite obscure, despite many years of experimental investigation with susceptible laboratory animals. In contrast, the susceptibility of the higher marine animals has been little studied.[1]

The Marine Biological Laboratory at Woods Hole provided facilities for a few experiments on eels, *Anguilla chryspa,* and dogfish, *Mustelus canis,* to determine their susceptibility to diphtheria toxin under different conditions. Inoculations were all intramuscular. An eel, weighing 106.5 gm., received 125 M.L.D. of diphtheria toxin. Nine days after inoculation, the eel became inactive but did not die until the twenty-fourth day. Two control eels, inoculated, respectively, with toxin broth and with heated diphtheria toxin, remained normal. Two other seawater eels were gradually habituated to fresh water. They weighed 40 and 50 gm. and were inoculated with 62 M.L.D. of the diphtheria toxin. Two days after inoculation, the eels were unusually active. This excessive activity subsided slowly and the animals became quiescent, breathing with difficulty. They both died on the thirteenth day, or in about half the time required by those in seawater.

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[1] Metchnikoff, Elie, Immunity in infective diseases, 1901. Translated by F. G. Binnie, 1907. London, Cambridge University Press.

A young seawater dogfish, weighing 1067 gm., was inoculated with 1375 M.L.D. of diphtheria toxin without effect. Four dogfish were gradually habituated to an environment of 50% fresh water. Two, weighing 681 and 818 gm. respectively, received series of doses ranging from 25 to 1250 M.L.D. of diphtheria toxin. The action of the toxin was manifest in about 7 days—the smaller animal died in 26 days, the larger in 20 days. The 2 control dogfish remained normal and increased in size.

At autopsy of both the inoculated eels and the dogfish, no tissue nor organs differed in any respect from those of the normal animal sacrificed for comparison.

The difficulties encountered throughout this work rendered it impracticable to include a larger number of eels and dogfish, and one can only record the results of this limited series of experiments, which consistently indicate susceptibility in the eel and, in comparison, an immunity in the seawater dogfish. The susceptibility of the eel and also that of the dogfish was apparently increased when their environment was changed wholly or in part to fresh water.

In view of these findings, it was obviously important to determine the action of seawater on diphtheria toxin. Accordingly, guinea pigs were inoculated with diphtheria toxin after it had been exposed to the action of seawater at room temperature. The results indicated that there is a somewhat greater loss in toxicity for guinea pigs in such diphtheria toxin than in that exposed to the action of a similar amount of 0.85% salt solution.

Immunization of eels and dogfish with diphtheria toxin was attempted in a few experiments. Neither the serum nor the plasma of any of these animals neutralized toxin when titrated on the guinea pig. The reactions induced by the eel sera alone, however, obscured the intracutaneous titration.

The toxicity of eel serum and dogfish plasma was tested on rabbits and guinea pigs. The numerous reports in the literature concerning the toxicity of eel serum for the rabbit were first confirmed: intravenous injection of 0.3 cc. of the seawater-eel serum proved fatal in 27 hours. When the sera of eels which had been gradually adapted to fresh water were tested, 0.3 cc. or even 1.25 cc. failed to prove toxic. The injections of skate plasma, reported in the literature as toxic,[2] gave rise to practically no symptoms in the rabbit. Intracutaneous injection of the guinea pig was also studied. The plasma of the dogfish and the skate, as well as seawater, incited practically no reaction except a slight redness. The sera of the seawater eel and of those habituated to fresh water proved toxic in that they gave rise to marked local reaction with necrosis. The serum of the eel habituated to fresh water appeared to be less toxic in these experiments.

The period of incubation after injection suggests a complex mechanism determining the action of toxin in the tissues. The action of diphtheria toxin in the seawater eel, as compared with its action in the guinea pig, is delayed. In fresh water the susceptibility of the seawater eel is increased; and the dogfish, ordinarily immune, becomes susceptible when transferred to an environment of half fresh and half seawater.

7006 C

Normal Emptying Time of the Stomach of the Dog.

EDWARD J. VAN LIERE AND GEORGE CRISLER.
(Introduced by A. J. Carlson.)

From the Department of Physiology, University of West Virginia.

In the course of experimental work on the effect of anoxemia on the emptying time of the stomach of the dog a good deal of control data was obtained. It was felt that these data might be of interest and use to other workers, especially as there appears to be a considerable difference of opinion as to the time it takes the stomach of a dog to empty.

Dogs varying in weight from 5 to 10 kilos were used in these experiments. They were fed approximately the same time each day. The experimental meal consisted of 40 gm. of hamburger steak, 10 gm. of dried ground bread and 50 cc. of milk; 15 gm. of barium sulphate were added so that the stomach contents could be seen with the x-rays. The animals ate this meal with relish. Immediately afterward they were put into a large steel respiratory chamber so that they would be protected from extraneous influences. The chamber was kept well ventilated by means of an air pump of large capacity. It was necessary, of course, to remove the animals from the chamber in order to examine them fluoroscopically. This procedure took from 5 to 15 minutes, so that an experimental error of a plus or minus 15 minutes was allowed.

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[2] Camus, Lucien, and Gley, E., *C. r. Soc. de biol.*, 1915, **78**, 203.

TABLE I

No. of dog	Average emptying time of stomach hr.	No. exp.
1	6.86	16
2	6.11	7
3	6.12	2
4	8.00	6
5	7.21	7
6	7.66	5
7	6.75	7
8	6.65	26
9	6.54	12
10	5.75	3
11	6.00	4
12	5.42	3
13	6.66	9
14	6.89	12
15	7.06	8
16	6.29	13
17	6.39	16
18	7.65	5
19	5.50	11
20	6.25	4
21	6.06	4
22	6.70	5
23	8.07	4
24	6.21	7
25	5.88	4

Summary. Twenty-five dogs were used; a total of 200 tests was run. The average emptying time of the stomach of the dogs was 6.59 hours; the mean was 6.54 hours. The average for the tests was 6.61 hours and the mean 6.62. The extremes for the emptying time were 5.42 hours and 8.07 hours.

It was interesting to observe that the normal emptying time of the stomach for an individual dog was remarkably uniform from day to day. It was necessary, of course, to keep all the factors quite constant. As a matter of fact the extremes for the 25 dogs as before mentioned were 5.42 hours and 8.07 hours—a variation of only 2.65 hours. No correlation was noticed as to size or breed.

The results reported in this paper are higher than those obtained by Ivy[1] and by Thomas.[2] The former reports the normal emptying time of the stomach in 5 dogs as varying from 4 hours and 28 minutes to 5 hours and 17 minutes. He used a meal of 125 gm. of Swift's Silver Fox Food and 100 cc. of milk. Thomas states that a dog fed about 300 gm. of dog biscuit and a pint of milk would probably empty in somewhere around 3 hours. Both of these authors used a different type of meal from the one reported in this paper and this probably accounts for the difference in results. The experimental meal we used proved very successful in our work and we can recommend it highly; it is fairly well balanced; the dogs ate it with relish and small dogs subsisted solely on it.

Conclusions. The normal emptying time of the stomach was determined on 25 dogs under carefully controlled conditions. The meal consisted of 40 gm. of hamburger steak, 10 gm. of dried ground bread and 50 cc. of milk; barium sulphate was added (15 gm.). A total of 200 tests were made. The average emptying time of the stomach of the dogs was 6.59 hours; the average for the tests was 6.61 hours. The variations were between 5.42 and 8.07 hours. It was found that the emptying time of the stomach for an individual dog was strikingly uniform from day to day.

7007

Effect of Massage on Blood Platelet Production.*

S. P. LUCIA AND J. F. RICKARD. (Introduced by Wm. J. Kerr.)

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Fenn[1] demonstrated the ease with which erythrocytes undergo hemolysis when they come in contact with glass. Davidson[2] showed that a relatively large proportion of erythrocytes contain basophilic staining-substance. Watson[3] concluded that "the fragments of

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[1] Ivy, A. C., and Fauley, G. B., *Am. J. Physiol.*, 1929, **91**, 206.

[2] Thomas, J. E., *J. Am. Med. Assn.*, 1931, **97**, 1663.

* Assisted by a grant from the Christine Breon Fund.

[1] Fenn, W. O., *J. Exp. Med.*, 1922, **35**, 271.

[2] Davidson, L. S. P., *Edinburgh Med. J.*, 1930, **37**, 425.

[3] Watson, C. H., *Edinburgh Med. J.*, 1932, **39**, 229.

effete or damaged erythrocytes correspond in every detail to the bodies known as blood platelets." For these reasons, we wish to record our observations on the blood platelets of the rabbit. During these experiments it was observed that there were marked variations in the platelet-counts from the same animals, and that the estimated counts from blood-smears did not agree with those calculated in the counting-chamber.

The blood platelets of normal adult rabbits were counted. Chamber-counts and smears were prepared from the same drop of blood obtained from the ear (a) without trauma, (b) after moderate trauma, and (c) after prolonged trauma. Variations between chamber- and smear-counts were studied, and the effects of time on the formation of platelets in the pipette were observed. The massage consisted of gentle, but firm, stroking of the ear at a rate of 25 strokes per minute for one minute and for 5 minute periods. A distal venous ramus of the ear was punctured and the first drop of blood withdrawn was studied. Anders Kristenson[4] fluid was used to dilute the blood. It was filtered and centrifugalized for 20 minutes immediately before each experiment. An erythrocyte-counting pipette was filled as follows: diluent to the 0.5 mark; blood to the 0.5 mark; then diluent to the 101 mark. The pipette was shaken for 3 minutes and a Levi-Neubauer counting-chamber was filled and allowed to stand for 20 minutes. Only smooth, rounded, highly refractile bodies were counted as platelets. The limit of accuracy by this method was 70,000 per cu. mm. Platelets were classified as "large" when 4 micra or greater, and "small" when less than 4 micra, in diameter. The large platelets appeared as clear, rounded, or ovoid bodies containing dark-staining, highly refractile granules. The smear stained by Wright's method was divided into 9 equal zones and the number of platelets per 1,000 erythrocytes in each zone estimated. The average for 9,000 red blood cells was multiplied by the total erythrocyte count expressed in thousands, in order to obtain the total platelet-count per cu. mm. of blood.

The following tabulation records a typical observation on a series of 5 rabbits. The figures express the average platelet count per cu. mm. of blood:

Effects of Massage on Platelet-Count

	Normal	After 1 min. Massage	After 5 min. Massage
Large	342,000	338,000	286,000
Small	540,000	906,000	434,000
Total	882,000	1,244,000	720,000

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[4] Kristenson, Anders, *Acta. Med. Scand.*, 1922-23, **57**, 301.

To show the discrepancy between chamber- and smear-estimation platelet counts, the pipettes were filled and the smears made from the same drop of blood. The following gives the average values for 13 such counts:

The Difference between Chamber- and Smear-Counts

Chamber-Count	Smear-Estimation
927,000	329,000

A third experiment demonstrates the effect of time on successive drops withdrawn from the same pipette at varying intervals of time. The pipette was thoroughly shaken before each successive count:

Effect of Time on Platelet-Count (Chamber-Method) from same Pipette.

	Normal	After ¾ hr.	After 1½ hrs.	After 4 hrs.
Large	200,000	240,000	270,000	190,000
Small	580,000	1,040,000	950,000	560,000
Total	780,000	1,280,000	1,220,000	750,000

Similar observations have been repeatedly made on the same and different animals. They vary only in the degree of initial platelet count, the general trend of each experiment being unaffected.

To control any variation in the platelet counts due to excitement, an animal was handled in the same manner as in the previous experiments, except that the ears were not traumatized. Platelet counts done before and after a one-minute interval do not show any significant variation.

Effect of Excitement on Platelet-Count

	Normal	One minute later without trauma
Large	170,000	100,000
Small	270,000	360,000
Total	440,000	460,000

To determine whether the change in platelet count of the traumatized ear was the reflection of a local or a general systemic change, a platelet count was done on blood obtained from an untraumatized ear. After one minute of trauma, platelet counts were made on blood obtained from the traumatized and opposite, untraumatized ears. The results follow:

Effect of Trauma on Platelet-Count of Opposite Ear

	Right ear before massage	Right ear after massage	Untraumatized left ear
Large	85,000	230,000	85,000
Small	360,000	460,000	218,000
Total	445,000	690,000	303,000

Summary. Moderate massage or trauma causes an increase in the total platelet count. After prolonged trauma, the count drops to normal. These phenomena occur locally in the traumatized ear. The counting chamber method of enumerating blood platelets gives values which are 2.8 greater than the smear estimation values. The platelet count, from the same pipette after increasing intervals of time, rises at first and then falls. Anders Kristenson fluid is undesirable for counting blood platelets because it dissolves erythrocytes.

7008 P

The Fixation of Certain Viruses on the Cells of Susceptible Animals and Protection Afforded by Such Cells.

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Rabbit embryo tissue was grown in a thin plasma clot and after some days the cultures were submitted to a trypsin solution strong enough to digest the clot and free the cells that had extended into it.[1] By repeated pipettings and filtrations through lens paper, alternated with differential washings in gelatin-Tyrode solution, suspensions were obtained of the living cells as individuals. They were mixed at room temperature with suspensions of virus, and after an interval the cells were recovered with the centrifuge, again washed repeatedly, treated in various ways and inoculated into rabbits. The viruses used were vaccinia and the filterable agent causing the rabbit fibroma described by Shope.[2]

Cells exposed to virus and repeatedly washed invariably gave rise to lesions in susceptible animals. The briefest exposure at room temperature permitted by the conditions resulted in an association of the virus with the cells, which withstood many washings of the latter. The fixation thus indicated took place not only upon living cells but upon those killed by heat, ultraviolet light, and water respectively, and often was as considerable. Incubation of the material with immune serum *in vitro,* followed by repeated washings prior to infection, resulted in neutralization of the virus associated with dead cells, whereas these procedures were without effect when the cells were alive. The possibility that serum antibodies accumulated on the dead cells and were carried into the final inoculum was ruled out by appropriate tests.

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[1] Rous, Peyton, and Jones, F. S., *J. Exp. Med.*, 1916, **23**, 549.

[2] Shope, R. E., *J. Exp. Med.*, 1932, **56**, 793.

Suspensions of individual, washed cells procured from cultures of Shope tumor tissue, or from rabbit embryo cultures inoculated with vaccinia, were found to carry the virus. When the cells were alive incubation with immune serum had no effect on the associated virus, whereas when they had been killed in one of the ways mentioned the virus was more or less completely neutralized by the serum. In cultures of Shope tumor tissue the virus seems to be strictly localized to the cells.

The experiments would appear to throw light on the process of infection with the viruses studied and they offer an explanation of some of the difficulties of serum treatment. A protection of bacteria by living cells has been demonstrated in a previous paper from this laboratory and its implications have been pointed out.[3] The cells of a chicken sarcoma protect the associated causative agent so well that growths frequently develop when they are transplanted to fowls having immune principles in circulation that are capable of neutralizing the agent as such.[4]

7009 P

Observations on Life History of a Gregarine of the Striped Shore Crab, Pachygrapsus crassipes.*

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The common striped shore crab, *Pachygrapsus crassipes,* of the Pacific Coast is frequently infected by a Cephaline gregarine which inhabits the mid- and hind-gut, the infection rate sometimes running from 70% to 100%. No correlation is apparent between the extent of infection and the locality or ecological habitat of the host, nor does there seem to be seasonal distribution of the infection.

Apparently, the entire life history of this gregarine takes place in

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[3] Rous, Peyton, and Jones, F. S., *J. Exp. Med.,* 1916, **23**, 603.

[4] Fischer, A., *Z. Krebsforsch.,* 1927, **14**, 580.

* Aided by a grant from the Board of Research of the University of California.

the lumen of the mid-gut and hind-gut of the host, no intra-cellular stages having been seen. Trophozoites, forms "in copula", encysting stages, and cysts may all be present at the same time in the digestive tract.

The classification of this gregarine depends largely upon the method by which the infection is transmitted from one host to another. In the family Porosporidae, the encysted gregarines are transferred in the form of a multinucleated cyst to a mollusk; this cyst invades the gills and produces infective spores for the crab. On the other hand, there are certain genera, such as *Uradiophora* and *Cephaloidophora* for which no intermediate hosts are necessary.

It is possible to obtain parasite-free crabs in the laboratory by starving and isolating them for 2 weeks. "Clean" crabs so obtained were kept alive in separate aquaria for several months by feeding them on a diet of chopped liver. The intestinal contents of infected animals were then fed to these clean crabs, and the latter were examined at intervals for gregarines. It was possible to transmit the infection from crab to crab; control animals captured at the same time but not fed infected material showed no parasites in their gut. This artificially transmitted infection proved in some cases to be exceptionally heavy. All of the various stages of this parasite could pass through the anterior portion of the digestive tract of the crab unharmed. Infection thus may be transmitted without the intervention of any intermediate host.

Crabs are ordinarily cannibalistic; and infection is probably transmitted from host to host, at least in part, in this way. Crabs kept together in an aquarium and not fed will prey on their weaker fellows. Such crabs retain their infections for considerably longer periods than do similar isolated ones. No infected animals have been found among those forms commonly associated with the crab, such as barnacles or mussels, which might be suspected as serving as possible intermediate hosts. Infection by the discharge of resistant stages into the sea water is uncommon, if it occurs at all normally, since the parasite is rarely found in the posterior part of the hind gut or in the feces of the crab.

The organism resembles most closely forms in the genus *Uradiophora* created by Mercier.[1] However, the incomplete life history and the confused taxonomy of the gregarines parasitizing the Crustacea do not warrant classifying this form at present beyond the Cephalina.

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[1] Mercier, L., *Arch. Zool. Exp. et Gen.*, 1912, **10**, 177.

The effect of parasitism on the crab seems to be limited to the destruction of the epithelial and cuticular lining of the gut by the mechanical action of the parasite. The epithelial lining may be thinned out beneath the point of attachment of the parasite, or in other cases may be sloughed off, especially in heavy infections. Since the gregarine does not appear to pierce the cells, it must obtain its food supply from the lumen of the digestive tract. Although an exceedingly heavy infection might destroy sufficient tissue to inconvenience the host, it is doubtful if this would occur normally since, even in severe infections, comparatively few of the host's epithelial cells seem to have been materially injured.

7010 C

Reticulocyte Counts in Bartonella Muris Anemia.

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The striking stimulation of the bone marrow in splenectomized rats suffering with *Bartonella muris* anemia is reflected in the reticulocyte count of the peripheral blood. In the course of extensive studies in this disease[1-7] the appearance of large numbers of reticulocytes during the height of the anemia was noted.* A detailed correlation of the degree of anemia as reflected in the blood count and hemoglobin determination with the reticulocyte count and the appearance of Bartonella bodies has yielded certain interesting observations. For the study and demonstration of reticulocytes in

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[1] Marmorston-Gottesman, J., and Perla, D., *J. Exp. Med.*, 1930, **52**, 121.

[2] Perla, D., and Marmorston-Gottesman, J., *J. Exp. Med.*, 1930, **52**, 131.

[3] Perla, D., and Marmorston-Gottesman, J., *J. Exp. Med.*, 1931, **53**, 869.

[4] Marmorston-Gottesman, J., and Perla, D., *J. Exp. Med.*, 1931, **53**, 877.

[5] Perla, D., and Marmorston-Gottesman, J., *J. Exp. Med.*, 1932, **56**, 777.

[6] Perla, D., and Marmorston-Gottesman, J., *J. Exp. Med.*, 1932, **56**, 783.

[7] Sandberg, M., Perla, D., and Marmorston-Gottesman, J., *J. Exp. Med.*, 1933, **57**, 81.

* For a review of *Bartonella muris* anemia see Lauda[8] and the papers of Perla and Marmorston-Gottesman, and the recent review of Kikuth.[9]

[8] Lauda, E., In Kolle, W., and von Wassermann, A., *Handbuch der pathogenen Mikroorganismen*, Jena, Gustav Fischer, 3rd ed. (Kolle, W., Kraus, R., u. Uhlenhuth, P.) 1930, **8**, Liefg, 20, 1073.

[9] Kikuth, W., *Ergebn. d. Hyg. Bakt. Immunitätsforsch. u. exp. Therap.*, 1932, **13**, 559.

large numbers, *Bartonella muris* anemia offers a readily obtained source.

Estimations of the hemoglobin, red blood cells and reticulocytes were made daily on a group of 6 splenectomized male adult rats of *Bartonella muris* carrier stock, from the day of splenectomy to the death of the rats. Hemoglobin was determined by the Dare colorimeter. For the preparations of the smears for the reticulocyte count a slight modification of the method recommended by Key[10] and Thoro[11] was used. The dye was diluted as follows: 4 drops of a saturated aqueous solution of brilliant cresyl blue were added to 5 cc. of physiological salt solution to which had been added 2 drops of a 2% potassium oxalate solution. This was made up fresh every few days. One drop of the dye solution was placed on the slide and near it one drop of blood. The blood and dye were intimately mixed with the edge of a second slide and smears drawn. When dry the smears were counterstained with Wright's stain. The reticulocytes were clearly defined and readily counted. Smears prepared without vital staining were made for the study of Bartonella bodies.

Stucky and Brandt[12] recently reported the curve of the reticulocyte count in normal rats at different ages. They found that the drop in the hemoglobin level during the nursing period is associated with a progressive decrease in the number of reticulocytes. At birth 60% of the red cells are reticulocytes. There is a progressive decrease after this. At 21 days the reticulocytes have dropped to a 25% level. At 2-3 months normal rats show a constant 2-3% reticulocyte count. We have been able to confirm these observations. In our own stock at 3 months of age, the reticulocyte count of normal rats varied from 1.7 to 4.8%.

From the table it may be seen that with the development of anemia 4 to 5 days after splenectomy a rise in the reticulocytes occurred to a level as high as 23%. As the anemia progressed and the hemoglobin and red cell count fell, the percentage of reticulocytes progressively increased. By the 8th day after splenectomy the reticulocyte count was over 40%. By the 10th day the animals began to recover, the hemoglobin rose, the red cell count increased and the reticulocyte count progressively diminished so that on the 22nd day the count dropped in most of the rats to below 10%. Two days later they again rose as the anemia recurred to as high as 70% and

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[10] Key, J. A., *Arch. Int. Med.*, 1921, **28**, 5.

[11] Thoro, W. C., Clinical Laboratory Methods. Press Publishing Co., East Stroudsburg, Pa., 1926, 53.

[12] Stucky, C. J., and Brand, E., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 932.

TABLE I

Reticulocyte Counts in Bartonella muris Anemia of Splenectomized Adult Albino Rats

Rat No.		Before Oper.	Counts After Operation—No. Days																	
			1	2	4	6	7	8	9	11	12	15	17	20	22	24	28	36	44	48
1	%Hbg.[1]	110	105	100	95	20	20	30	35	60	55	80	90	115	60	80	80	90	70	
	R.B.C. × 000	8500	8600	7700	6200	1300	2200	1500	2000	3200	4950	5400	6500	6400	3200	4200	5500	7000	4500	Died 48th day
	% Retic.[3]	3.2	3	6.4	4	15.6	29	50	33	30	10	8	6	2	7	22	8	1	2	
	% Bart. m[4].	0	0	0	30	8	R	R	R	0	R	R	R	2	25	0	0	0	R	
2	% Hgb.	105	108	95	100	90	70	35	35	25	40	70	75	105	85	90	55	85	40	
	R.B.C. × 000	8000	8200	8500	8100	7500	5000	1150	2500	3400	3200	4000	5400	5600	4450	4250	4550	6000	3200	" 47th "
	% Retic.	3.6	1.6	4	3	1.2	40	8	16	20	18	18	21	3	6	40	72	33	56	
	% Bart. m.	0	0	0	0	0	50	3	2	R	R	0	R	12	15	1	0	0	0	
3	% Hgb.	110	110	100	95	10														
	R.B.C. × 000	9000	8600	7200	5600	1400														
	% Retic.	2	1.6	3	3.4	3	Died													
	% Bart. m.	0	0	0	0	90														
4	% Hgb.	106	100	110	85	10	30	35	40	55	105	95	50	20	35	55	90	40	90	
	R.B.C. × 000	8000	8600	6600	5400	2500	2800	2500	2900	3350	4650	5500	4050	2150	1000	2700	4600	2000	6200	" 46th "
	% Retic.	4.8	1	2.6	16	40	42	40	26.4	22	23	18	23	50	36	38	18	65	4	
	% Bart. m.	0	0	0	88	7	R	R	R	0	0	2	30	2	0	0	20	10	6	
5	% Hgb.	95	95	90	40	25	40	80	90	80	120	100	100	10						
	R.B.C. × 000	8500	8600	6700	5300	1800	3000	2550	4400	5450	6100	4500	5500	2700						" 22nd "
	% Retic.	3.7	4.4	2	23	39	47	43	33	25	22	15	5	3						
	% Bart. m.	0	0	0	75	R	R	0	R	0	R	R	R	10						
6	% Hgb.	105	115	100	85	40	25	50	45	110	100	90	70	100	105	85	45	50	40	
	R.B.C. × 000	9000	8700	6500	6800	2500	2500	3000	3750	4850	5550	5150	4500	5550	5250	4850	4900	3500	3000	" 45th "
	% Retic.	1.7	5.4	1	12.8	38	25	41	50	52	56	52	55	23	10	6	50	36	12	
	% Bart. m.	0	0	0	95	84	R	R	0	0	4	0	0	0	5	5	8	5	15	

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[1] The Hemoglobin expressed in % as calculated from readings with the Dare Hemoglobinometer.

[3] % of reticulocytes determined by counting 500 red blood cells.

[4] % number of red cells containing Bartonella bodies indicated in this column.

R. Rare.

continued high till just prior to death when they dropped considerably. A persistently low reticulocyte count in the presence of continued anemia is a fatal prognostic sign.

Concomitantly with the rise in the reticulocyte count and the drop in hemoglobin percentage and the number of red cells, there was a marked decrease in the percentage of red cells and the number of red cells infected with Bartonella bodies. The height of the Bartonella infection of the red cells occurs just before the marked drop in hemoglobin percentage and the red cell count. With the development of a severe anemia, Bartonella bodies were found with difficulty, though when the anemia recurred the appearance of large numbers of Bartonella bodies again was manifest in the early phase of the relapse.

The resistance of young red cells (reticulocytes) to *Bartonella muris* is striking. In the vitally stained preparations Bartonella bodies were never observed within the reticulocytes. The complete disappearance of Bartonella bodies at the height of the anemia suggests a peculiar resistance of the new formed red cells to their invasion. This factor may account for temporary recovery from the infection that follows the anemia in most instances. In a large percentage of instances, however, particularly in males, relapses occur and death of anemia and infection result.

Conclusions. 1. Reticulocytes may constitute more than two-thirds of the red cells in the circulation at the height of the anemia in *Bartonella muris* anemia of splenectomized adult albino rats. 2. Reticulocytes are particularly resistant to invasion by *Bartonella muris*. 3. The reticulocyte response during *Bartonella muris* anemia may be used as a prognostic sign. A poor reticulocyte response is associated with death of the rat within a few hours. On the other hand a high reticulocyte count is followed by temporary recovery from the anemia though the rat may succumb during a subsequent relapse.

7011 C

Comparative Effects of Dinitrophenol and Thyroxin on Tadpole Metamorphosis.*

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Since a fundamental action of thyroid is an increase in the metabolic rate, it might be supposed that such metabolic stimulation might be responsible for the well known acceleration of metamorphosis in tadpoles.[1] If this were true, the rate of metamorphosis might be increased by dinitrophenol (1-2-4), which has been shown to increase mammalian metabolism to a remarkable degree.[2] However, the results of this study indicate that, in this respect, these 2 metabolic stimulants differ, as indeed they do also clinically.[3]

Tadpoles of *Bufo halophilus* from the same and neighboring egg strings and in the same stage of development were selected at random from a swarm of about 1,000 and divided into 13 groups of 10 each. Two groups were used as untreated controls, 3 for various concentrations of thyroxin, and 8 for various concentrations of alpha-dinitrophenol (1-2-4). Each group of 10 was kept in 200 cc. of tap water which contained a clump of green algae and about 5 cc. of pond water. The solutions and algae were changed every second day. Later in the experiment, 1 cc. of raw minced liver was fed each group every second day, about an hour before changing the solutions. Solutions of crystalline thyroxin (E. R. Squibb and Sons) were added to the baths to give final concentrations ranging between 1:150,000 and 1:2,000,000, and of dinitrophenol to give final concentrations ranging from 1:5,000 to 1:4,000,000. The pH of all the final solutions, including the controls, was between 7.21 and 7.47.

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* Supported in part by a grant from the Rockefeller Fluid Research Fund of the School of Medicine, Stanford University.

[1] Gudernatsch, J. F., *Arch. f. Entwich. Mech. d. Organ.*, 1912, **35**, 475.

[2] Cutting, W. C., and Tainter, M. L., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1268. Tainter, M. L., Boyes, J. H., and DeEds, F., *Arch. Int. de Pharm. et de Therap.*, 1933, **45**, 235. Cutting, W. C., Mehrtens, H. G., and Tainter, M. L., *J. Am. Med. Assn.*, 1933, **101**, 193. Tainter, M. L., and Cutting, W. C., *J. Pharm. Exp. Therap.*, 1933, **48**, 410. Tainter, M. L., and Cutting, W. C., *J. Pharm. Exp. Therap.*, 1933, **49**, 187. Hall, V. E., Field, J., Sahyun, M., Cutting, W. C., and Tainter, M. L., *Am. J. Physiol.*, in press. Cutting, W. C., and Tainter, M. L., *J. Am. Med. Assn.*, in press. Tainter, M. L., Stockton, A. B., and Cutting, W. C., *J. Am. Med. Assn.*, in press.

[3] Cutting, W. C., Rytand, D., and Tainter, M. L., in press.

This close similarity in the pH values is important, since Zondek and Ucko[4] have shown that the increase in rate of metamorphosis in tadpoles by thyroid extract is modified by the H-ion concentration of the medium. The experiments were made at a fairly constant room temperature of about 22°C. and in dull sunlight. Records of the growth and development were made at frequent intervals and accurate measurements of length were made by photographing the tadpoles against a millimeter scale.

Results. The average initial length of the tadpoles was 9.5 mm., the external gills were still present and the heads were slender and oval. In the stronger concentrations of dinitrophenol the tadpoles swam excitedly and incessantly until death, often colliding with each other. All the members of these groups died at about the same time; those in 1:5,000 dinitrophenol within 30 minutes, in 1:10,000 within 1½ hours, and in 1:50,000 within 12 hours. In the weaker concentrations of dinitrophenol, the external gills, which were present on the first day, entirely disappeared on the second day and the slender oval heads gradually became more rhomboid and blunt, the eyes more prominent and the stomach pouchy.

In 5 days, the tadpoles in 1:150,000 thyroxin were definitely smaller than those in any of the control or dinitrophenol groups, and 3 of the 10 tadpoles in this thyroxin-group showed hind limb buds. In 7 days, the hind limb buds were well developed on all tadpoles in 1:150,000 thyroxin and also on 3 tadpoles in 1:500,000 thyroxin. The tails showed evidences of deformity and the mouths were wider and strikingly more jaw-like than the sucking mouths still present in the tadpoles of the other groups. In the latter, the eyes were less prominent and heads less rhomboid than in the tadpoles on thyroxin. On the 10th day, there were evident the first fore limb buds, these in one tadpole on thyroxin 1:150,000. By this time, the hind limbs were beginning to differentiate into upper and lower legs, and toes appeared. Deaths at this stage were frequent in the 1:150,000 thyroxin, but none occurred in the other groups. Although no hind limb buds had appeared in either the control or the dinitrophenol groups, these tadpoles were definitely larger and stronger than their metamorphosing neighbors on thyroxin. On the 20th day, the first limb buds appeared in the control and dinitrophenol groups, one being present in the controls and one in 1:4,000,000 dinitrophenol. On the following day, one such bud appeared in 1:500,000 dinitrophenol. At this time, all tadpoles on thyroxin had well developed hind limbs which were one-fourth to one-half the tail length.

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[4] Zondek and Ucko, *Klin. Wochschr.*, 1924, **3**, 1752.

One early fore limb bud was present in thyroxin 1:500,000. On the 24th day, all the tadpoles in 1:2,000,000 thyroxin had well developed hind limbs and 2 had early fore limb buds. One control and one tadpole in each of 1:500,000 and 1:4,000,000 dinitrophenol had early hind limb buds.

Tadpoles in thyroxin 1:500,000 were completely metamorphosed by the 30th day, and those in thyroxin 1:2,000,000 were metamorphosed by the 50th day, while only 30% of those on dinitrophenol and 20% of the controls had even limb buds as yet. By the 70th day, only 60% of both control and dinitrophenol groups showed hind limb buds, but no fore limbs at all. The characteristic curve of metamorphosis shown by the tadpoles on thyroxin and the striking similarity between the unmedicated and dinitrophenol groups are illustrated in Fig. 1.

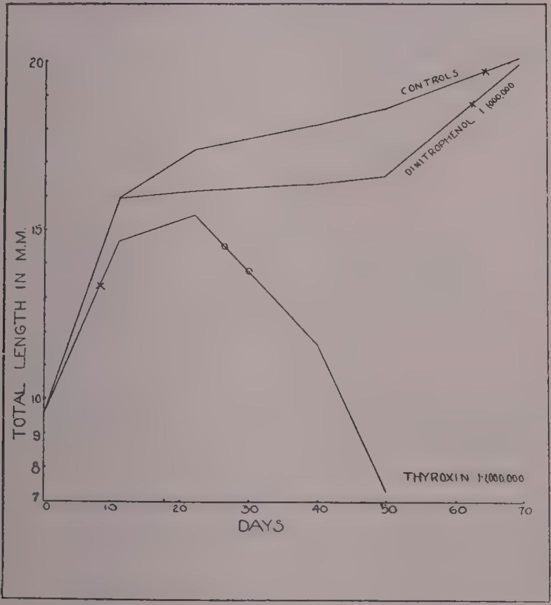

FIG. 1.

Effects of thyroxin and dinitrophenol on growth and metamorphosis of tadpoles. Each curve shows the average changes in length of 10 tadpoles in a given solution. The times at which 50% of the tadpoles developed hind limb buds are indicated by crosses, fore limb buds by a square, and complete atrophy of the tail by a circle.

Another experiment was made on the same species of tadpoles, about one week after leaving their jelly strings. The procedure was the same as in the previous experiment, except that dried grated liver was used for feed throughout. The thyroxin used was a solution containing 1 mg. per cc. in ampules (Roche; N. N. R.) diluted freshly to a 1:1,000,000 solution with tap water. It seemed to be more toxic to the tadpoles, so that weaker concentrations were necessary. Otherwise, the results agreed entirely with those described above and hence need not be repeated.

Conclusions. 1. Toad tadpoles were found to metamorphose no more rapidly in up to lethal concentrations of dinitrophenol than in the absence of medication, whereas the usual acceleration of metamorphosis was demonstrated in tadpoles on thyroxin. This difference in action agrees with clinical differences in human subjects. 2. Although dinitrophenol can cause increases in metabolism comparable to those of thyroxin, it appears to lack the power of thyroxin to accelerate developmental processes. Apparently, the effect of thyroxin on metamorphosis is not the direct result of increased metabolism, but presumably an independent action. 3. Accordingly, dinitrophenol lacks the hormonal actions of thyroid, and should probably not be used therapeutically to replace the gland in true thyroid deficiencies.

7012 C

Effect of Arm Compression on Local Venous Pressure in Patients with Normal and Abnormal Hearts.*

R. C. LEVY AND W. A. BRAMS. (Introduced by L. N. Katz.)

From the Medical Department of Cook County Hospital, the Cardiovascular Group, Michael Reese Hospital, and the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.

It is well known that interference with the flow in a vein will cause a rise in venous pressure within that vessel. Villaret, Saint-Girons and Justin-Besançon,[1] and others, have observed that mediastinal tumor or aortic aneurysm may lead to an elevation in venous pressure in one arm if its veins are obstructed. Runge[2] found a higher pressure in the saphenous vein than in the arm veins during pregnancy; a difference which disappeared after delivery. A similar difference was found by Villaret, Saint-Girons and Justin-Besançon[1] in cases of ascites or abdominal tumor. Brams, Katz and Kohn[3] noted a marked rise in pressure in the iliac veins with little or no change in the superior vena cava in experimentally induced abdominal disten-

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* Aided by the Frederick K. Babson Fund of the Michael Reese Hospital.

tion in animals. Brams and Katz[4] observed that slowly increased compression of a vein in animals produced a gradual rise in venous pressure up to a certain level, after which little or no further change occurred.

In this investigation the effect of experimental compression was studied in man to determine: (a) whether or not the results are the same in persons with normal and failing hearts; and (b) what relation the maximum rise in venous pressure bore to the systolic and diastolic arterial blood pressures.

Six subjects with normal hearts and normal blood pressures were selected as controls. Two patients with mediastinal tumor and 18 with varying degrees of cardiac failure comprised the group of abnormals. Cardiac failure in the latter was due to rheumatic fever, hypertension, cor pulmonale or arteriosclerosis. Venous pressure was measured by the direct method, using a hollow needle inserted into the median basilic vein after the patient rested under basal conditions for at least 15 minutes as described by Eyster. The needle was connected to a suitable mercury manometer since, in preliminary trials, it was found that compression of the vein produced a rise in venous pressure too great to be registered by an ordinary water manometer. The mercury manometer was found to be sufficiently sensitive for the needs of the present research. An ordinary sphygmomanometer cuff was used to compress the arm cephalad to the point where the needle was inserted into the veins. This was gradually inflated in steps and the pressure in the cuff and in the vein noted at each step.

A study of the results in the normal persons showed a progressive rise in venous pressure on increasing compression of the arm, similar to that seen in the previously reported animal experiments.[4] No change in the slope of the rise of venous pressure occurred when the compression force on the arm approached the diastolic arterial pressure. A definite change in the curve of venous pressure occurred in all instances when the compression force approached systolic blood pressure. Further compression, above the systolic pressure, resulted in a fall of venous pressure varying from 2 to 40 mm. of mercury. This last level was maintained practically constant on further compression of the arm.

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[1] Villaret, M., Saint-Girons, F., and Justin-Besançon, L., "La Pression Veineuse Périphérique," Masson and Cie, 1930, Paris.

[2] Runge, H., *Arch. f. Gynak.*, 1924, **122**, 142.

[3] Brams, W. A., Katz, L. N., and Kohn, L., *Am. J. Phys.*, 1933, **104**, 120.

[4] Brams, W. A., and Katz, L. N., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 98.

The 2 patients with mediastinal tumor showed similar results. The 18 patients with varying degrees of cardiac failure reacted in the same manner, the fall in venous pressure ranging from 10 to 80 mm. of mercury when the compression force exceeded the systolic arterial pressure level.

The gradual rise in venous pressure with compression by the cuff was due to accumulation of blood in the vein peripheral to the point of obstruction. The abrupt fall in venous pressure when compression reached or slightly exceeded the systolic blood pressure can be explained by assuming that practically all the flow in the arteries beyond the point of compression was stopped. The contents in the distended veins could now regurgitate into their "proximal" capillaries and collapsed arteries since the pressure in the latter had fallen close to zero. Such regurgitation would continue until the pressure became equal in the veins and arteries below the cuff. The constant level of venous pressure after this drop is easily understood since all the vessels of the limb below the cuff are practically isolated from the rest of the body. The fact that no difference was observed in persons with normal and abnormal hearts can be explained by the fact that the previously described changes were due to local factors at the point of compression.

Summary. 1. A series of 26 observations were made in man to study the effect of gradually increased compression on the venous pressure below the point of compression. This group consisted of 6 normal persons serving as controls and an abnormal group of 2 with mediastinal tumor and 18 with cardiac failure. 2. Venous pressure gradually rose as compression was increased, until the latter reached the level of the systolic arterial pressure. At that point or on slightly further compression the venous pressure fell from 2 to 80 mm. of mercury and remained unchanged at that lower level regardless of the degree of further compression. 3. The progressive rise and the drop at the systolic pressure level were found in all the groups of patients regardless of the actual level of the systolic blood pressure or the condition of the heart. No change in the slope of the venous pressure rise was seen when the compression force approximated the diastolic blood pressure.

7013 C

Skin Changes after Subcutaneous Injection of Epinephrine.

M. CAROLINE HRUBETZ. (Introduced by H. B. Williams).

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It is taught that epinephrine is much less effective by hypodermic or intramuscular injection than by the intravenous route. These differences are due to the rate of absorption, and this in turn to the vasoconstriction (Sollmann[1]). The unabsorbed epinephrine at the site of injection is apparently not destroyed, for the local blanching may persist from one to 8 hours (root of rabbit's ear, Auer and Meltzer,[2] Meltzer and Auer[3]; scarified skin, Sollmann and Pilcher[4]). When this epinephrine becomes gradually absorbed, it exerts its systemic actions. (Fornet,[5] Lyon[6]). It follows therefore, that if epinephrine were administered by a route such that it be slowly absorbed, its action would not be as sudden as by the intravenous route.

During an experiment in which epinephrine was injected subcutaneously in the rat, a considerable percentage of the animals showed skin changes at the site of injection, ranging from loss of hair to necrosis and, in a few cases, gangrene. As the experiment included the use of various doses, a record of the animals was kept with respect to size of the dose of epinephrine and degree of skin change.

The epinephrine was diluted with isotonic sodium chloride so that 1 cc. contained 0.4 mg. The full dose given was 0.1 mg. per 250 gm. of rat. In the case of the ½, ¼, ⅛ doses, the proper dilution was made. Abbott's epinephrine hydrochloride was used throughout the experiment.

Table I shows the relative changes with the various doses.

TABLE I

Dosage	No. animals	% showing only loss of hair	% showing breakdown of skin tissue	% showing no reaction
Full Dose	361	54	13	34
½ Dose	46	39	30	30
¼ Dose	169	5	0	95
⅛ Dose	30	—	—	100

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[1] Sollmann, T., A Manual of Pharmacology, 437.

[2] Auer, J., and Meltzer, *J. Pharm. Exp. Therap.*, 1917, **9**, 358.

[3] Meltzer and Auer, *J. Pharm. Exp. Therap.*, 1921, **17**, 177.

[4] Sollmann and Pilcher, *J. Pharm. Exp. Therap.*, 1917, **9**, 309.

[5] Fornet, B., *Arch. f. exp. Path. u. Pharm.*, 1922, **92**, 165.

[6] Lyon, M., *J. Exp. Med.*, 1923, **38**, 655; *Quart. J. Med.*, 1923, **17**, 19.

The question arose as to whether these changes were due to the vasoconstrictor effect of the epinephrine or the acid which it contains. The acidity of our sample was determined by means of the hydrogen electrode. A solution of the corresponding strength of HCl was made and amounts equivalent to the quantities contained in the full dose of epinephrine were given subcutaneously. Forty-two rats were used. Twelve showed very slight hair loss but in no case, any signs of necrosis. It seemed, therefore, that the extensive skin effects were due to the epinephrine itself.

Patta[7] extracted the skin and subcutaneous tissue 2 hours after injection. He obtained a marked elevation of blood pressure upon intravenous injection of this extract, and concluded that epinephrine is not destroyed beneath the skin, at least within 2 hours. Luckhardt[8] injected, subcutaneously, 3 cc. of 1:1,000 epinephrine in the dog. Forty-nine pressor responses were obtained by the massage of this area. After 9½ hours, a saline extract was made from the subcutaneous tissue and a rise of 150 mm. of mercury was obtained as a result of its intravenous injection. He has obtained pressor effects from massage 19 hours after the subcutaneous injection of 1 cc. of 1:1,000 adrenalin HCl. Even after that time a saline extract of the subcutaneous tissue exerted, on intravenous injection, a marked pressor effect. He makes the significant statement that "the rate of absorption in the dog and in man must be different because in the dog, the subcutaneous injection of 1 cc. was followed in 19 hours by a moist gangrene of the injected area." Hare[9] states that he had seen cases where adrenalin had been given hypodermically and sloughs occurred.

We find that it is accepted by surgeons that such a skin effect may occur in man. At the Willard-Parker Hospital of New York City, instructions are given for massage of the injected area whenever itching recurs after the subcutaneous injection for serum sickness, etc. One injection will keep a patient comfortable from 12 to 15 hours.

Lederle Laboratoies issue a recommendation with their epinephrine for the massage of the injected area 24 to 48 hours after injection in order to obtain a second effect. Parke-Davis issues a similar recommendation.

In conclusion, it appears that epinephrine is not easily destroyed in the skin. It is probable that the tissues are sufficiently buffered to prevent the rapid decomposition of the drug.

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[7] Patta, *Arch. d. Pharm.*, 1905, **4**, 329.

[8] Luckhardt, A. B., *Am. J. Physiol.*, 1927, **81**, 436.

[9] Hare, *Trans. Assn. Am. Phys.*, 1904, **19**, 233.

7014 C

Growth Inhibiting Agent in Extracts of Desiccated Mammary Gland.

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From the Department of Pathology, Loyola University School of Medicine.

Hormones, notably thyroxin, insulin, parathormone and epinephrine, have been shown to inhibit proliferation of the glandular tissues that produce them.[1, 2, 3, 4] The availability of these hormones in concentrated form made possible a determination of their effect on the mitotic rate of the glands. The histological methods for calculating rates of proliferation in the endocrine organs are equally applicable to tissues that do not produce internal secretions. In this laboratory aqueous extracts of desiccated kidney were found to inhibit the proliferation of the epithelium of the renal tubules when injected intraperitoneally into young rats.[5] Saline extracts of desic-

TABLE I

Mouse No.	CC. Ext. Injected	Mitoses per 10 mm.^{2} 24 hrs. after injection	at time of injection
1	1.2	505.5	772.3
2	1.4	244.0	387.5
3	2.2	58.5	271.8
4	2.0	4.1	345.3
5	2.4	60.6	66.4
6	2.2	250.6	1161.5
7	1.8	166.7	245.5
8	2.0	162.9	381.4
9	2.2	21.1	188.2
10	2.0	262.8	334.6
11	2.2	378.4	408.6
12	2.0	433.5	722.1
13	2.0	59.5	121.3
14	2.2	111.3	468.0

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[1] Loeb, L., *J. Med. Res.*, 1920, **41**, 481.

[2] McJunkin, F. A., and Roberts, B. D., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 893.

[3] McJunkin, F. A., Tweedy, W. R., and Breuhans, H. C., *Arch. Path.*, 1932, **14**, 649.

[4] McJunkin, F. A., Rall, R. R., and Singer, P. L., *Endocrinol.*, 1932, **16**, 635.

[5] McJunkin, F. A., and Hartman, C. D., *Am. J. Path.*, 1933, in press.

cated beef lactating mammary gland were made by the same method and injected into mice with spontaneous cancers of the mammary gland.* Tumor tissue was obtained by biopsy at the time of injection and the mitoses counted. Twenty-four hours later the animal was killed and the rate of proliferation determined. The results are shown in Table I. The experiments show that the extracts prepared from the mammary gland inhibit mitoses in the adenocarcinomata developing spontaneously in mice mammary glands. The mitoses in the tumors of control mice subjected to ether anesthesia and the same operative procedure were reduced not at all or to a much less degree. The inhibitory effect was seen about 24 hours after the injections and subsequently the tumors continued to grow at their usual rate.

7015 C

Gall Bladder Function after Pancreatectomy.

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In the course of an extended series of observations on depancreatized dogs a number of questions arose in the interpretation of the data. Many other changes in body economy besides loss of insulin and loss of juice in the intestine have to be considered. Some questions have arisen concerning altered biliary function.

The following experiments were performed in order to determine the functional activity of the gall-bladder in dogs after complete pancreatectomy. For this purpose the dye method of Graham, Cole and Copher[1] was employed in 3 dogs which had been depancreatized 173, 242 and 287 days respectively. The animals were kept on a diet consisting essentially of cooked lean meat and glucose, supplemented by insulin.

The experiments were conducted in the following manner: 0.15 gm. of sodium tetraiodophenolphthalein (iodeikon) per kilo body weight was injected intravenously. Eighteen hours afterward an X-ray of the gall bladder was taken. Then the dogs were given their ration of meat and glucose to which 40 gm. of beef suet was added, and insulin was injected. Two hours and 5 hours after the feeding cholecystograms were made to study the emptying time of the gall bladder.

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* The strain of mice was obtained from M. C. Marsh, Springville, N. Y.

[1] Graham, E. A., Cole, W. H., and Copher, G. H., *J. Am. Med. Assn.*, 1925, **84** 1175.

The results were as follows: 18 hours after the injection of the dye, well defined cholecystograms were obtained in all of the dogs, demonstrating good concentration. After feeding, the shadow of the gall bladder diminished in size and density or disappeared completely, indicating partial or complete removal of the dye. These results are well within the range of the variations found in the functional activity of the gall bladder in normal dogs and demonstrate that removal of the pancreas does not alter the function of the gall bladder as determined by the dye method.

7016 C

Phosphatase Studies. IV. Serum Phosphatase of Non-osseous Origin. Significance of the Variations of Serum Phosphatase in Jaundice.

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From the Laboratory Division, Hospital for Joint Diseases, New York City.

In view of the wide occurrence of phosphatase in other tissues than bone, the possibility of non-osseous origin of *some* of the serum phosphatase deserves examination. It receives support from the evidence of the great decrease of serum phosphatase after prolonged fasting[1] and its great increase after ingestion of dextrin.[2] The significance of phenomena of normal physiology has frequently been suggested by a disturbance of the function of a given organ or tissue; we believe that the increase of serum phosphatase in jaundice is of similar importance and indicates that liver is a source of serum phosphatase, normally as well as pathologically.

The procedure for determination of serum phosphatase has been described.[3]* The icteric index was determined by Meulengracht's method.

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[1] Bodansky, A., and Jaffe, H. L., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 199.

[2] Bodansky, A., unpublished data.

[3] Bodansky, A., *J. Biol. Chem.*, 1933, **101**, 93.

* About 2.6 and 7.5 units of phosphatase per 100 cc. are the average normal figures for adults and children, respectively (normal range for adults, 1.5 to 4.0 units; normal range for children, 5.0 to 13.0 units).

Results. Serum phosphatase was increased in about 50 observed cases of jaundice, *with the exception of some cases of anemia.*

In acute catarrhal jaundice bile pigments elaborated in the liver, and phosphatase, presumably of the same origin, were found in the serum in increased quantities. Yielding readily to treatment, this condition was particularly suitable for a demonstration of the decline of the serum phosphatase from its high initial values (Table I). Other clinical conditions associated with jaundice and high serum phosphatase were not equally suitable for a study from the physiological standpoint.

TABLE I

Serum Phosphatase in Treated Cases with Catarrhal Jaundice and Hepatitis.

No.	Age	Sex	Per 100 cc. serum Phosphatase units	Icteric Index	Notes	Days later
1	10	F	19.0	57	Infect. catarr. jaund.	10
			9.2	13		
2	12	F	23.8	20	" " "	
			13.6	7		6
			9.7	5		7
3	7	F	22.8	27	" " "	
			13.3	13		6
			9.9	6		7
4	5	F	13.1	8	" " "	
			10.1	7		4
5	9	F	10.4	58	Acute " "	
			7.6	7		11
6	8	F	22.1	20	" " "	
			8.0	Normal		15
7	60	M	18.5	13	" " "	
			10.0	10	" " "	5
8	9	M	9.8	33	" " "	
			6.6	13		12
			4.8	6	" " "	10
			6.9	10	" " "	53
9	17	M	14.9	>50	Subacute hepatitis	
			12.6	9		10
			8.1	8		8

The decrease of the icteric index during treatment corresponded with the clinical improvement. The figures in Table I show that serum phosphatase decreased with the icteric index. It may be noted in Case 8 that not only did serum phosphatase decrease as the case improved, but that it increased during relapse.

Robison has stated that the production of bone phosphatase formed "a part of those cellular activities leading to the formation of bone,"[4] and Kay demonstrated the increase of plasma phosphatase in certain bone diseases.[5] It has been held since that increase of plasma phosphatase "is confined almost exclusively to cases of bone disease,"[1] although Roberts had demonstrated increased plasma phosphatase in jaundice,[6] and plasma or serum phosphatase has been stated to be almost exclusively of osseous origin.

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[4] Robison, R., *Ergebnisse der Enzymforschung,* 1932, **1**, 280.

[5] Kay, H. D., *J. Biol. Chem.,* 1930, **89**, 249.

It seems timely to point out the hepatogenous origin of some of the serum phosphatase as indicated by the high serum phosphatase found in jaundice (with the exception of some cases of anemia) and by the decrease of serum phosphatase in the course of successful treatment of catarrhal jaundice and hepatitis. The seeming exception of normal serum phosphatase figures in cases of jaundice with anemia confirms our view and emphasizes the hepatogenous rather than the hematogenous factor in jaundice as a source of increased phosphatase. It may be assumed, furthermore, that some of the serum phosphatase originates in the liver even under normal conditions.

7017 P

Factors Influencing Rate of Reduction of Potassium Ferricyanide by "Resting" Escherischia coli.

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It has been shown[1] that broth cultures of *Staphylococcus aureus* in the maximum stationary phase reduce potassium ferricyanide at a rate in the neighborhood of 6.6×10^{-14} millimols per cell per minute. Further work with cultures of *Escherischia coli* (K-12) gave similar results and will be reported elsewhere. The experiments to be reported here were designed to measure quantitatively the rate of reduction of potassium ferricyanide by "resting" *Esch. coli* under carefully controlled environmental conditions.

Forty-eight hour cultures of *Esch. coli*, grown on nutrient agar at 37.5° C., were suspended in saline and washed 3 times by centrifugation. The organisms were then diluted with a mixture of equal parts of M/15 phosphate buffer, pH 7.5, and physiological saline solution to a standard turbidity corresponding to a total count of approximately 20×10^9 cells per cubic centimeter.

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[6] Roberts, W. M., *Brit. J. Exp. Path.*, 1930, **11**, 90.

[1] Clifton, C. E., *J. Bact.*, 1933, **25**, 495.

Measured volumes of this suspension of "resting" *Esch coli* were introduced into tubes similar to those employed in the studies with *Staph. aureus*[1] and appropriate amounts of buffer, saline, and Difco peptone solutions were added. These suspensions were deaerated for one-half hour, by bubbling nitrogen through them at a constant rate, before the addition of a 0.1 molar solution of 3 parts of potassium ferricyanide to 1 part of potassium ferrocyanide. The change in the redox potential with time at 37.5°C. was followed potentiometrically at 10 minute intervals and the concentration of ferricyanide calculated directly from the potential readings.

The rate of reduction of the ferricyanide increases with increase in concentration of peptone (other variables being maintained constant) up to 2.0 or 3.0% and then decreases as the concentration of peptone is further increased. The rate of reduction of ferricyanide by "resting" *Esch. coli* in the absence of peptone or by peptone in the absence of "resting" bacteria is negligible. Duplicate tests agree within 1.0% and similar tests with suspensions prepared at different times give results of the same order of magnitude.

The number of millimols of ferricyanide reduced per cell per minute, in a 1% peptone solution, decreases as the concentration of bacteria is increased. Under the same conditions the rate of reduction of the ferricyanide increases as the concentration of the ferricyanide is increased. Similar results were obtained at pH 7.0 and pH 8.0. A decrease in temperature of 15°C. reduced the rate of reduction of the ferricyanide to approximately one-half of the value observed at 37.5°C.

The bearing of these results on the general problem of bacterial growth and behavior will be discussed in the final paper. On the basis of a considerable number of tests as summarized above we may, however, at present conclude that the concentration of peptone, organisms, and oxidant play important and closely connected rôles in controlling the metabolic activities of the cells in the average bacterial culture.

7018 P

Development of Somatic Activity in the Albino Rat Fetuses.

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From the Wistar Institute of Anatomy and Biology, Philadelphia.

The earliest reflex activity elicited by light tactile stimulation in the albino rat has been described.[1] Further investigations have been made upon the fetuses described as non-motile in that contribution. These fetuses do not respond to light tactile stimuli or to the fixative (chemical stimulation). The younger fetuses of this period are also irresponsive to ligation of the umbilical cord (endogenous stimulation). However, the majority of the fetuses of the non-motile stage responded to strong stimulation applied by means of a porcupine quill when stimulated in the neck and fore-limb region. The responses consisted of lateral flexion of the head towards the side stimulated and extension of the fore limbs respectively. Also, some of the older fetuses of this period responded to endogenous stimulation with lateral flexion of the head only. It is evident from these observations that somatic activity, other than that elicited by light tactile stimuli, develops at an earlier period than that described in my previous paper. In addition, these observations show that the younger fetuses responded only to strong mechanical stimuli and the older fetuses responded to both strong mechanical and endogenous stimulation. The responses in respect to these 2 means of stimulation are distinctly different in quality, that is, the contractions elicited by endogenous means are slow, feeble and quickly followed by relaxation; while those elicited by strong mechanical stimuli are also slow and feeble, but maintained. A very long latent period was noticed when strong mechanical stimulation was used.

The fetuses of the early motile stage responded to light tactile stimulation of the snout only, to endogenous stimuli, and to strong mechanical stimulation applied with a porcupine quill. Here again the response differs in relation to the means of stimulation used. The light touch stimulation upon the snout elicited responses of lateral flexion of the head away from the side of stimulation. The endogenous stimulation elicited response of lateral flexion of the head, and often this contraction spread caudad so as to involve the fore limbs in this movement. These contractions were also slow, feeble, and quickly followed by relaxation. The strong mechanical stimulation elicited responses similar to those obtained in the previous stage. However, the responses to stimulation of the neck region with the quill were towards the side stimulated, while those elicited with light tactile stimulation of the snout were away from the side stimulated. Fetuses of the 19 day stage were also tested in the fore limb region with strong mechanical stimuli. Even after the definite establishment of reflex activity in this region,[1] the fetuses responded to this type of stimuli in the same characteristic manner, except that the contractions were maintained for a much longer period, and the latent period was a great deal shorter than in the younger stages.

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[1] Angulo y Gonzalez, A. W., *J. Comp. Neur.*, 1932, **55**.

The method employed for the investigation of the latency of period is too crude to withstand critical quantitative analysis. However, the following general ideas have been obtained that may be useful as a point of departure for further investigation: The latent period increases in a cephalo-caudal and proximo-distal direction. Within certain limits it decreases with the strength of stimuli, and it also decreases with the age of the fetuses.

The foregoing observations indicate that there are 3 phases in the development of somatic activity in the albino rat fetuses. The movements of the first phase are believed to be myogenic in nature, elicited by direct strong stimulation of the muscle. Other means of stimulation fail to elicit response. The long latent period observed in these cases might be construed as indicative of neurogenic activity. However, the undeveloped striated muscle has a long latent period (31.6 sigma for premature baby).[2] Therefore, the exceedingly long latent period obtained in the above experiments cannot be used as indicative of neurogenic activity. The second phase in somatic activity seems to be purely neuro-motor in nature, indicated by the fact that responses were obtained endogenously where tactile and chemical stimuli had failed. The third phase is sensory-motor nature, that is, reflex activity in the strict sense.

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[2] Krasnogorski, N., *Jahrb. f. Kinderh.*, 1914, **79**.

7019 C

Existence of Micellae in Aqueous Solutions of Saponin.

WILLIAM KLEINBERG. (Introduced by Eric Ponder.)

From Washington Square College, New York University.

It is well known that if certain surface active substances are adsorbed into a surface to form a monolayer, there are certain critical concentrations in which the static surface tension is at a minimum, these corresponding to the concentrations in which the molecules at the surface form regular patterns. Granted certain assumptions, it is possible to determine from the values for these critical concentrations the dimensions of the molecule of the surface active substance, and in this way du Nouy[1] has determined the mean length, breadth, and thickness of such molecules as that of sodium oleate and serum albumin.

This note concerns the result obtained when similar experiments are carried out with saponin in aqueous solution. The technique of du Nouy was followed in detail, dynamic and static surface tension measurements being made on solutions of quillia saponin contained in Petrie dishes with a surface area of 63.6 cm.[2] Each dynamic surface tension measurement is the average of 15 separate determinations, and each static measurement the average of 10 determinations made after the solution has remained undisturbed for 2 hours. In the calculation, the density of saponin was taken as unity.

The graph shows that well marked minima occur in the static tension at concentrations of 1 in 12,500, 1 in 24,500, and 1 in 37,500, and the question arises as to what the appearance of these minima means. If we suppose that they are the result of an orderly arrangement of molecules in a monolayer, we get for the dimensions of the saponin molecule 6.3×10^{-6}, 3.2×10^{-6}, and 2.1×10^{-6} cm., which, considering the molecular weight of saponin (in the neighborhood of 1000) are absurdly large. The presence of the minima is nevertheless unmistakable, (the minima being just as definite as those obtained by du Nouy for sodium oleate) and if the method is to be trusted at all, the dimensions which are calculated from the values for the critical concentrations must therefore represent the dimensions of orderly arranged agglomerates: not molecules, but micellae together, perhaps, with imbibed water, which are capable

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[1] P. Lecomte du Nouy, A. C. S. Monograph Series No. 27, 1921.

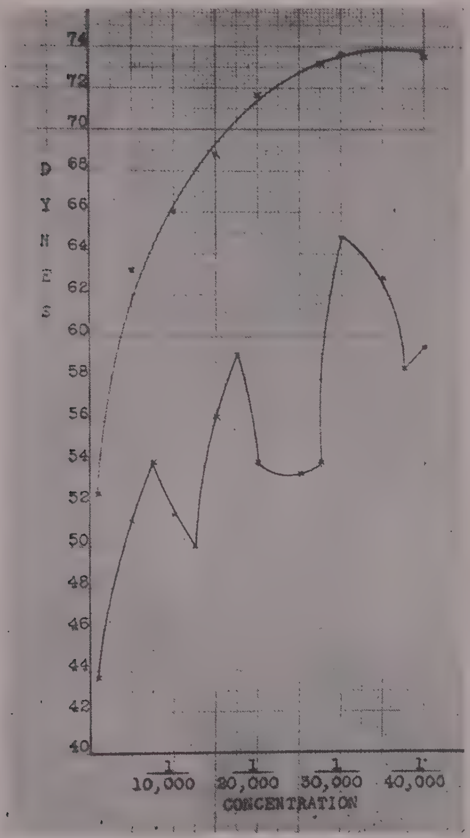

FIG. 1.

of orienting themselves in 3 different ways corresponding to their mean length, breadth, and thickness. The sharpness of the minima, moreover, suggests that the scatter about these mean values is not very great. As the dimensions of these micellae are about 10 times what one would expect for the saponin molecule itself, each agglomerate must contain about 10 molecules.

The concept of regular micellae, though seemingly novel, is in reality that of a liquid crystal formation.

7020 P

Reduction Intensity in Anaerobic Ameba dubia.

BARNETT COHEN AND T.-T. CHEN.

From the Department of Physiological Chemistry, Johns Hopkins Medical School.

Lack of suitable indicators has hitherto prevented an examination of intracellular oxidation-reduction intensities by microinjection under anoxybiosis. Earlier attempts by Cohen, Chambers and Reznikoff[1] using phenosafranine were inconclusive because the dye underwent irreversible change during the time required for manipulation and observation. Stiehler, Chen, and Clark[2] and Stiehler[3] have recently made available some new indicators for the more negative regions of reduction potential which we have utilized. Some of these compounds undergo irreversible change but by quick operation (injections made within a few minutes after reduction) we have succeeded in obtaining definite indications.

Observations were made in an improved hermetic chamber through which flowed moistened purified nitrogen or hydrogen. Injection of oxidizing agent or exposure to air were used to check the reversibility of the indicator in the cell interior. Results on immersion of the cells in oxidant or reductant confirmed those obtained by microinjection.

The experiments were restricted to one type of unicellular organism, *e. g., Ameba dubia.* The results are given in the accompanying table. These preliminary observations are now being augmented by examination of other available indicators.

TABLE I

Indicator	E'_0 at pH 7 (Volt)	Oxidant in cell	Reductant in cell
Dimethylphenosafranine	-0.260	Partly reduced	Reoxidized
Safranine T	-0.289	" "	"
Sulfonated rosindone, No. 6 (cf. Stiehler)	-0.380	Not "	"

To the Biological Laboratory at Cold Spring Harbor, L. I., and the Director, Dr. R. G. Harris, grateful appreciation is extended for the adequate facilities made available for this work.

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[1] Cohen, B., Chambers, R., and Reznikoff, P., *J. Gen. Physiol.*, 1928, **11**, 585.

[2] Stiehler, R., Chen, T.-T., and Clark, W. M., *J. A. C. S.*, 1933, **55**, 891.

[3] Stiehler, R., Dissertation, 1933, Johns Hopkins University.

7021 C

Blood Flow to the Kidney and Creatinine Clearance.*

GRACE MEDES AND J. F. HERRICK.

From the Department of Medicine, University of Minnesota Medical School, and the Division of Physics and Biophysical Research, The Mayo Clinic and The Mayo Foundation, Rochester.

In a series of experiments carried out at the University Hospital, Minneapolis, by one of us (Medes) in collaboration with Dr. Hilding Berglund, it was demonstrated that creatinine clearance may be elevated in hyperthyroidism. In 20 cases of hyperthyroidism the clearance averaged 196 ± 8.6 cc. per minute, as compared with a normal average of 171 ± 2.9 cc. per minute. In the former group the range extended from 111 to 332 cc. per minute, whereas in the latter group the highest value found in 60 tests was 236 cc. per minute. That increased blood flow through the kidneys might be one factor responsible for the increased creatinine clearance seemed probable and the present investigation, undertaken at the suggestion of Dr. Berglund, is an attempt to evaluate this factor.

The thermo-stromuhr method of Rein[1] for measuring blood flow was used because it permits observations to be made under more nearly normal physiologic conditions.[2, 3] All observations were made on dogs and limited to one kidney, the other having been removed 3 or more weeks prior to the measurement of blood flow. The unit was applied to the renal artery, employing the usual ether anesthesia and aseptic technic. After the recovery of the animal observations were begun. The observations were made at intervals of about 15 minutes, and the average value throughout the period was estimated.

Creatinine clearance was measured by the method of Rehberg.[4] Creatinine (1.5 to 2 gm., depending on the weight of the animal) was administered intravenously one hour before readings were started and 150 to 250 cc. of water was given by stomach tube. All urine was removed by catheter, the bladder was flushed with several washings of physiologic sodium chloride solution and the washings were added to the urine. Samples of blood were taken at the beginning and end of each period and the values were averaged. Plasma creatinine was determined by Folin's method. Unless the standard and unknown were of approximately the same concentration, the determination was repeated. Creatinine readings

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* Work done at the Institute of Experimental Medicine, The Mayo Clinic, Rochester, Minnesota.

[1] Rein, Hermann, Z. f. Biol., 1928, **87**, 394. In Abderhalden, Emil, *Handbuch der biol. Arbeitsmethoden.* Berlin, Urban and Schwarzenberg, 1929, **5**, 692.

[2] Baldes, E. J., Herrick, J. F., and Essex, H. E., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1109.

[3] Herrick, J. F., and Baldes, E. J., *Physics*, 1931, **1**, 407.

[4] Rehberg, P. B., *Zentralbl. f. inn. Med.*, 1929, **50**, 367.

TABLE I
Creatinine clearances and blood flow

Creatinine clearance control exper. cc. per min.	Days	Observation min.	Blood flow, cc. per min.	Creatinine clearance cc. per min.	Blood flow, % of maximum	Clearance, % of maximum
Dog 1. Wt. 9.7 kg.						
	1	38	90	19.1	83	80
22.0, 21.1, 24.7		43	104	22.9	96	96
Aver.—22.6		43	105	23.3	97	97
		50	106	23.5	98	98
		46	108	23.9	100	100
		14¾	90	13.9	83	58
		18¼	101	21.9	94	92
Dog 2. Wt. 14 kg.						
	1	45	261	37.2	97	100
37.4, 36.2, 36.0,		80	245	33.1	91	89
36.6		40	243	33.6	90	90
Aver.—36.6		77	269	37.3	100	100
		47	269	36.4	100	98
		60	269	36.3	100	97
		42	269	36.1	100	97
Dog 3. Wt. 11.3 kg.						
	1	40	130	37.5	90	89
42.1, 37.3, 37.6		41	133	39.0	92	93
Aver. —39.0		36	143	41.0	99	98
		35	144	41.5	100	99
		30	144	42.0	100	100
Dog 4. Wt. 4.6 kg.						
	1	48	115	12.4	63	75
		35	139	14.2	76	86
26.8, 27.6, 29.4,		32	140	15.2	77	92
27.9		38	165	16.1	90	98
Aver.—27.9		37	183	16.5	100	100
	2	50	295	18.9	100	100
		38	185	16.1	63	85
		48	192	17.1	65	90
		56	287	18.6	97	98
		43	190	16.5	64	87
		44	190	16.5	64	87
		49	198	17.5	67	93
Dog 5. Wt. 12.7 kg.						
	1	53	78	0.8	47	
		34	97	3.5	58	27
		28	128	9.8	77	76
		30	128	9.8	77	76
30.7, 34.3		31	167	12.9	100	100
Aver.—32.5	2	57	152	4.1	53	33
		61	244	9.2	85	75
		48	263	10.4	91	85
		44	263		91	
		40	263	10.5	91	85
		55	288	12.3	100	100

were corrected from a curve constructed, using the same set of reagents.

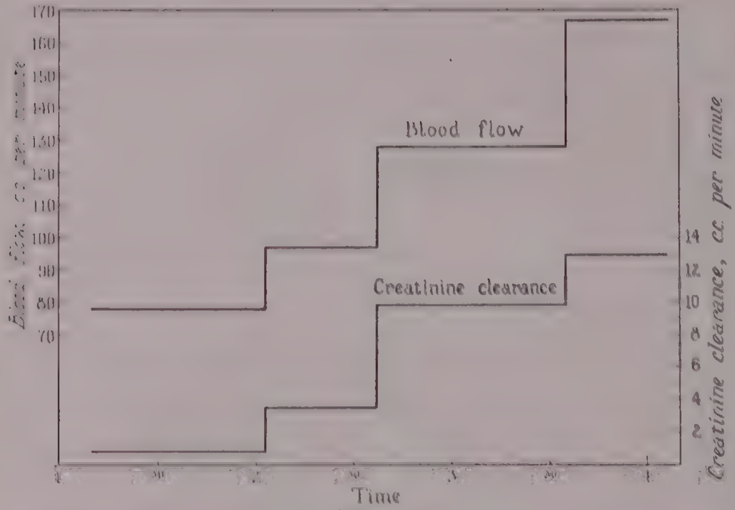

Fig. 1.

Parallelism between creatinine clearance and blood flow. The changes are typical for the first day of observation.

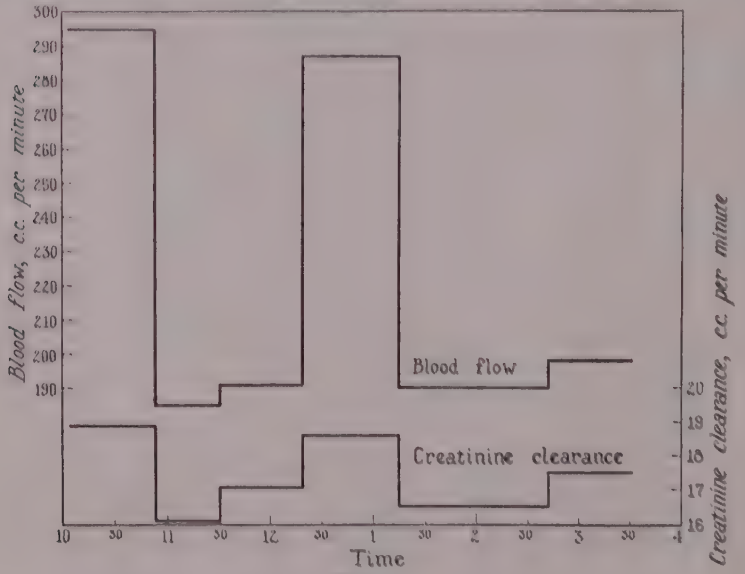

Fig. 2.

Parallelism between creatinine clearance and blood flow. The second day of an experiment. The increase between 12:00 and 1:00 follows a period of exercise.

At the close of the experiment the animal was killed, the position of the unit checked and the renal artery and kidney were examined grossly for possible injuries. In questionable cases sections of the kidney were examined microscopically.

A general parallelism has been found to exist between the blood flow and creatinine clearance (Table I, Fig. 1 and 2). Where the creatinine clearances approximate the values formed during the control periods (Exp. 1, 2 and 3) there seems to be almost a direct proportionality. However, the changes in blood flow were slight. In cases in which the fluctuations in blood flow were large and in which the creatinine clearances failed to return to their preoperative level, there is no direct proportionality (Fig. 1 and 2).

Observations made on the day of the operations showed a gradual increase in blood flow and creatinine clearance (Fig. 1). When the dogs recovered slowly the first period of observation showed a condition of marked oliguria with a scarcely measurable creatinine clearance and an appreciable blood flow. This instance of minimal creatinine clearance, in spite of an appreciable blood flow is in contrast to another observation (not recorded in the table): Creatinine clearance and blood flow diminished simultaneously and ultimately ceased during the second day of the experiment when the dog suddenly became nauseated. On exploration, it was found that the artery had become obstructed because of displacement of the unit applied to the blood vessel, which probably occurred during the retching accompanying vomiting. In this case in which the oliguria was caused by occlusion of the artery, both blood flow and creatinine clearance were measurable until anuria occurred.

Summary and Conclusions. A series of experiments was undertaken to study the relation between creatinine clearance and blood flow. The thermo-stromuhr method of measuring blood flow was used because it permits such observations without the complicating factor of anesthesia. All observations were made on unilateral nephrectomized dogs. A general parallelism but not a direct proportionality between the blood flow to the kidney and the creatinine clearance was observed.

7022 P

Influence of Environmental Change upon Sensitivity of Drosophila Larvae to Ultraviolet Radiation.

WILLIAM R. THOMPSON AND ROBERT TENNANT.
(Introduced by Raymond Hussey.)

From the Department of Pathology, Yale University School of Medicine.

We have developed in this laboratory a respirometer[1, 2, 3] capable of use under any conditions of constant humidity, and a technique of maintenance of drosophila larvae over periods of several days for respiratory studies. The technique and food ordinarily used is described in a report[4] of a study of immediate and subsequent effects of X-ray irradiation upon CO_2 respiration of larvae. In studies with ultraviolet light no significant effect upon duration of the prepupal period had been observed such as the extension[5, 6, 7] when X-rays were employed; but it seemed worth while to investigate the possibility that similar effects upon respiration might be obtained. Some striking similarities in the action of these two types of radiation were observed,[8] following which we were able to develop a means of affecting the sensitivity of the larvae to ultraviolet radiations.

Six random lots* of larvae (C_1, C_2, C_3, I_1, I_2, I_3) were maintained simultaneously as in the X-ray experiments mentioned above[4] except additions of glacial acetic acid were made to the regular 25 ml. of food as follows:

0.5 ml. for C_1 and I_1
1.0 ml. for C_2 and I_2 and
1.5 ml. for C_3 and I_3, each.

Respiration of CO_2 was observed as usual and the lots designated by *I* were irradiated simultaneously with the radiations from a mercury arc in quartz for ½ hour in a stream of air at 26° C. when they had attained a median age of approximately 3.5 days. The

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[1] Thompson, W. R., *J. Gen. Physiol.*, 1932, **16**, 5.

[2] Thompson, W. R., and Tennant, R., *J. Gen. Physiol.*, 1932, **16**, 23.

[3] Thompson, W. R., and Tennant, R., *J. Gen. Physiol.*, 1932, **16**, 27.

[4] Unpublished.

[5] Hussey, R., Thompson, W. R., and Calhoun, E. T., *Science*, 1927, **66**, 65.

[6] Tennant, R., *Science*, 1931, **73**, 567.

[7] Hussey, R., Thompson, W. R., Tennant, R., and Campbell, N. D., *J. Gen. Physiol.*, 1932, **16**, 207.

[8] Unpublished.

* Several hundred larvae were used in each lot, approximately 300 to 500.

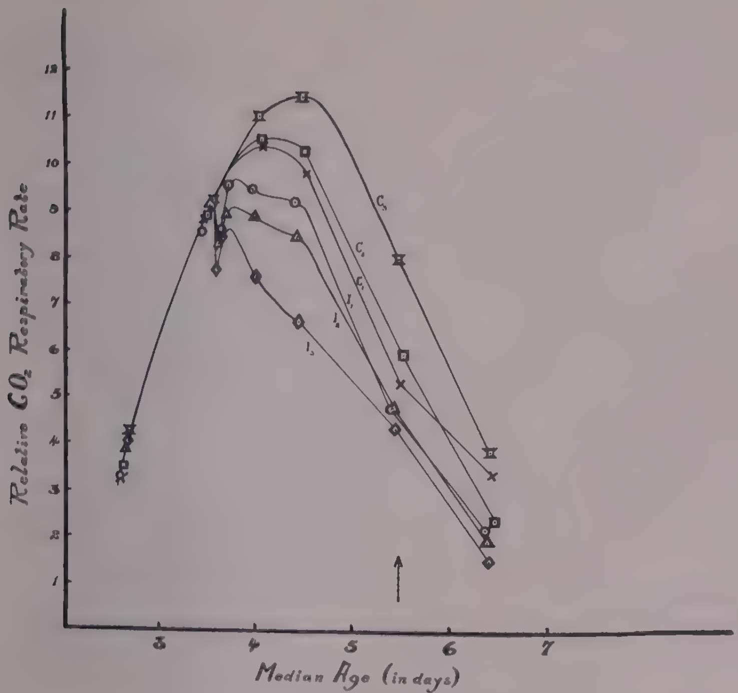

FIG. 1.

results are presented graphically in Fig. 1, where the ordinates of all curves are proportional to CO_2 respiratory rate, but in the case of each lot multiplied by a constant in order to superimpose their early portions. However, the ordinate scale approximately represents 10^{-8} moles per minute. The abscissae represents time in days during the experiment (integral values at midnight), and approximately the median age of the drosophilae. The median age at pupation (P) was sensibly the same in all cases and is indicated by an arrow on the chart. That sensitivity to irradiation increases with increase in acetic acid concentration is obvious here, as in the case of another experience in which, however, I_3 and C_3 were omitted. We hope to extend these studies to include similar observations on relative sensitivity to other forms of radiation such as X-rays.

7023 P

A Possibility of a Sympathetic Innervation of the Fish Heart.

FLOYD J. BRINLEY. (Introduced by A. J. Goldforb.)

From the Marine Biological Laboratory, Woods Hole.

The heart activity in all vertebrates is regulated by the nervous system and in the phyla above the fishes the heart is under the control of augmentor and inhibitory nerves. In the Teleost fishes there is an abundance of evidence that the vagus exhibits a powerful control over the heart. McWilliam,[1] Mills,[2] Bottazzi,[3] Armstrong,[4] and Brinley[5] have shown that the hearts of various fishes can be arrested either by direct or reflex vagal stimulation. On the other hand, there is no positive evidence that the heart of the Teleost fishes is innervated by the sympathetic system. Bottazzi[3] found that stimulation of the sympathetic system produced no effect on the heart rate. Brücke[6] stated that there is no evidence that the fish heart is controlled by accelerator fibers. However, Izquierdo[7] has shown that the heart of the shark, *Scyllium*, is under the control of the vagus and sympathetic nerves much the same as in the other cold blooded vertebrates.

The work of Izquierdo opens the question as to whether or not there is a sympathetic innervation of the Teleost heart. In connection with a study on the effect of drugs on the fish heart, a study was made on the action of substances known to stimulate the sympathetic system of higher vertebrates, on the embryonic and adult fish heart, to determine if the cardio-regulator nerves contain accelerator fibers. It is realized that no drug is strictly specific in its action on the nerves of the cardiac apparatus but it is believed that adrenalin and nicotine in proper concentrations approach this condition to a fair degree. This paper is concerned only with the action of adrenalin on *Fundulus heteroclitus* embryos.

A minute droplet of adrenalin chloride, from 1 part in 1000 to 1 in 50,000 distilled water was injected into Fundulus embryos (free from chorion) anterior to the vitalline arteries. The immediate contraction of the melanophores on the dorsal surface of the embryo was a positive criterion of a successful injection. Injections were made into embryos at daily intervals from 4 days until hatching. Controls were conducted by injecting distilled water into the embryos and in no case did any change occur in the rate of contraction except when large amounts were given. The rate of cardiac contractions was determined by counting the number of beats per minute before and after injection of the drug.

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[1] McWilliam, J. A., *Proc. Roy. Soc. London*, 1884, **38**, 31.

[2] Mills, T. W., *J. Phy.*, 1886, **7**, 82.

[3] Bottazzi, F., *Cent. für Physiol.*, 1901, **14**, 665.

[4] Armstrong, P. B., *J. Exp. Zool.*, 1931, **58**, 43.

[5] Brinley, F. J., *Phy. Zool.*, 1932, **5**, 527.

[6] v. Brücke, E., *Winterstein's Hand. der vergl. phy.*, 1925, **1**, 1007.

[7] Izquierdo, J. J., *J. Phy.*, 1930, **69**, 29.

Young embryo. Injections into embryos from 4 to 7 days old inclusive, do not produce any change in the rate or rhythm of the heart. This indicates that adrenalin has no visible effect on the rate of contraction of the embryonic cardiac muscle.

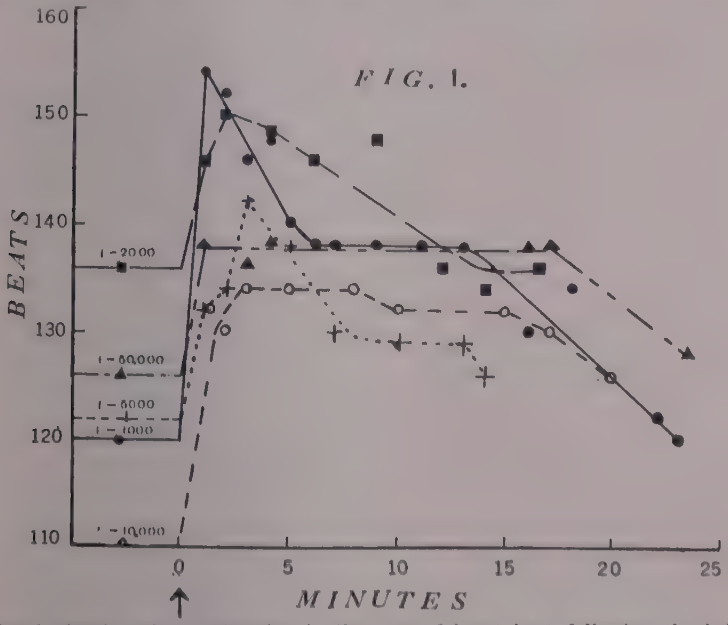
FIG. 1.

Graph showing the acceleration in the rate of heart beat following the injection of adrenalin chloride into Fundulus embryos 8 to 13 days after fertilization. Injections made at the time indicated by the arrow. The number of beats per minute is plotted as the ordinate and the abscissa indicates the time in minutes after injection.

Older embryos. Fig. 1 shows the results obtained by injecting adrenalin into embryos 8 to 13 days old inclusive. The heart of embryos 12 to 13 days old stops for several seconds immediately after injection which is due to a reflex stimulation of the vagus. Following this arrest and in all other cases the heart rate quickly increases 10 to 20% above the rate prior to injection and gradually returns to its initial value. This marked acceleration seems to indicate that the heart is either innervated by augmentor nerves on the eighth day after fertilization or that the cardiac muscle of the older embryo is decidedly different physiologically from the younger embryos as no effect of the drug is shown on the cardiac muscle prior to the 8th day of incubation.

To test the above hypotheses, adrenalin was injected into embryos which had previously been immersed for 30 minutes or longer in nicotine solution, pure alkaloid 1 part in 1000 parts of 5/8 normal (isotonic) NaCl solution. Nicotine in general is considered to be a sympathetic ganglionic depressant and in this concentration nicotine did not produce any marked action on the contraction of the embryonic heart muscle.

The results of these experiments show that injection of adrenalin after nicotine does not produce any significant acceleration in the cardiac rhythm. Therefore, it seems apparent that the acceleration in rate of the older embryos after adrenalin injection is due to a stimulation of the accelerator fibers and not to a direct action on the cardiac muscle.

7024 C

Transudation Through Living Membranes.

RAYMOND RATNOFF. (Introduced by Eric Ponder.)

From Washington Square College, New York University.

Gunn[1] has devised a mechanical system for transuding liquids through living membranes. The regulation and maintenance of a definite negative pressure in this system is based upon the method of Wade and Merriman[2] employing a variable air gap. Using this apparatus, Gunn tried the effect of the transudation of caffeine citrate and of Echis venom on rabbit mesentery. The results were negative in that no change in rate was observed with these substances when compared with the rate obtained with Locke's solution itself.

The apparatus has been simplified and improved, and in its final form provided for a complete glass assembly. (Fig. 1.) Using this improved apparatus I have carried out experiments to determine the effect of various lysins and of a series of chloride salts (Kahlbaum) upon transudation.

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[1] Gunn, J. A., *J. Physiol.*, 1931, **71**, 412.

[2] Wade and Merriman, *J. Chem. Soc. London*, 1911, **99**, 984.

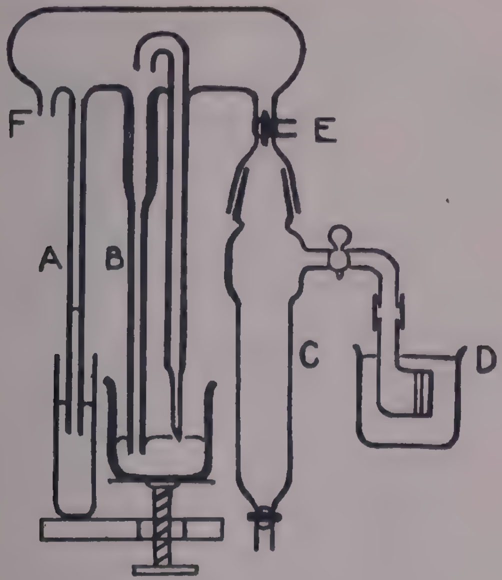

FIG. 1.

Diagram of apparatus. A, manometer; B, variable mercury air gap system; C, graduated burette for receiving transuded liquid; D, tambour in transuding solution; E, opening to air, usually closed; F, outlet to suction pump. The negative pressure in the apparatus is varied by adjusting the screw under B.

Living mesentery of freshly killed rabbits was used as the membrane. The procedure is essentially that of Gunn. The piece of mesentery, which had been previously placed in Locke's solution, is tied over the muslin-covered opening of a special tambour and transudation is begun after the tambour is fastened in place on the apparatus. To obtain a "normal curve" transudation is continued for 15 minutes under a pressure of 150 mm. Hg. at 26°C. Using a standard Locke's solution the transudation rate falls steadily with time. The beaker of Locke's solution is replaced by another containing Locke's solution to which has been added the desired concentration of lysin or salts to be tested. Transudation is continued and a new curve for the rate is obtained. Comparing the curves it is then possible to determine the change in transudation rate and thus the relative porosity of a given piece of mesentery.

I. SAPONIN. Transudation for 15 minutes in ordinary Locke's solution yields the "normal" hyperbolic transudation curve (Fig. 2).

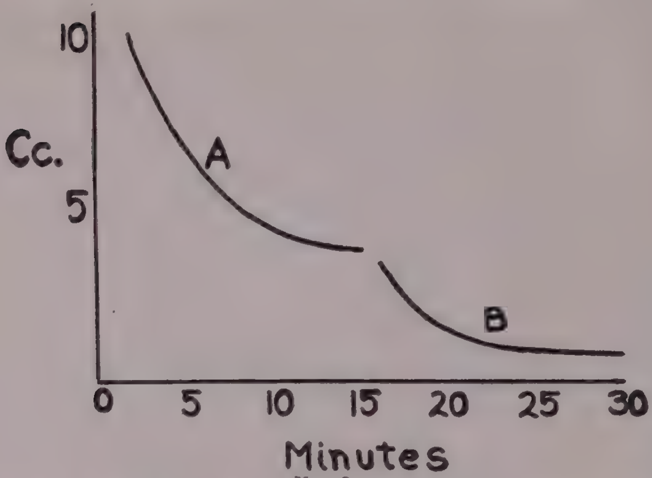

Fig. 2.

Ordinate, cc. of fluid transuded per min.; abscissa, time. Curve A, the normal transudation curve with Locke's solution; curve B, the curve when saponin, 1 in 1000, is added. The sudden decrease in transudation rate is apparent.

Upon following this by transudation for an additional 15 minutes with Locke's solution containing 1-1000 saponin there is observed, in every case, a decrease in transudation rate, in some cases small, in others quite large. The rate may drop from 3.5 cc./minute with Locke's solution to less than 1 cc./minute with saponin solution. It is as well to explain that while transudation curves of Locke's solution given by various pieces of mesentery are characteristically similar, yet, in disagreement with Gunn, I have found that the rates per minute vary greatly from mesentery to mesentery. In fact the rate for the first minute may vary from as little as 1 cc./minute to as much as 70 cc./minute.

II. Sodium Taurocholate. Sodium taurocholate in 1-1000 dilution in Locke's solution always causes a lowering of the transudation rate. In several cases the rate falls from 3.2 cc./minute with Locke's solution to less than 0.6 cc./minute with sodium taurocholate solution.

III. Alcohols. Methyl and butyl alcohols (0.5 N) both decrease the transudation rate. Ethyl and propyl alcohols (0.5N) affect the transudation rate in various ways; in some cases, the rate is increased, in others no effect is apparent, and in still others the rate is decreased. The changes are never very notable. Isopropyl alcohol has no effect.

IV. Chlorides. In order to obtain somewhat comparable conditions it is necessary to add such concentrations of the various salts as exert equal osmotic pressures. The amount added to one liter of Locke's solution was 11.3 gm. for NaCl, 14.0 gm. for KCl, 30.4 gm. for $BaCl_2$, 14.4 gm. for $CaCl_2$, 7.3 gm. for LiCl, and 25.7 gm. for $MgCl_2.6H_2O$.

Both NaCl and $BaCl_2$ in these dilutions give definite increases in the transudation rate. KCl, on the other hand, causes a definite decrease. The results for $MgCl_2$ and LiCl are variable. $CaCl_2$ causes slight changes in transudation rate varying from a slight increase to a slight decrease.

V. GLUCOSE. Glucose in concentration of 68.7 gm. per liter of Locke's solution brings about no significant change.

The rate of transudation for the first minute is always very high and as transudation is continued the rate gradually decreases. (Fig. 1.) Several experiments were carried out in which transudation was stopped and recommenced after an interval. After a "rest" from transudation the subsequent flow was higher than would have resulted had the flow been allowed to continue.

Although the results are not very striking, they are sufficient to indicate that certain substances (*e. g.*, haemolysins) which increase the permeability of red cell membranes, decrease the transudation flow through mesentery. It is to be borne in mind, of course, that permeability and transudation are not measured in exactly comparable terms. Salts and alcohols affect transudation in various ways and to different extents, and do not seem to fall into any series or relation to be expected on the basis of present theories of permeability.

7025 C

A Reply to Bishop and Heinbecker's "Fiber Distribution in Optic and Saphenous Nerves."

E. A. BLAIR AND JOSEPH ERLANGER.

From the Physiological Department, Washington University School of Medicine, St. Louis.

In a paper by Bishop and Heinbecker[1] there occurs a serious misinterpretation of a statement published by us.[2] They quote from our paper as follows, "The results therefore fail to support the view (of Bishop and Heinbecker), that there are fiber types distinguishable by time to maximum, conduction rate, irritability and refractory period. . . . . Since it is possible to recognize differences in the physiological responses of individual axons, it is no longer incumbent upon physiologists to adhere to the doctrine of specific nerve energies."

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[1] Bishop, G. H., and Heinbecker, P., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1312.

[2] Blair, E. A., and Erlanger, J., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 728.

These two passages they gratuitously take to mean "that the *same fiber* mediated (mediates) different sensations by means of *impulses* of *different character*" (italics theirs) and then proceed to attack, with facetiousness as their weapon, the windmill they have erected. As a matter of fact, we had no intention of conveying any such meaning and presented no evidence in support of such a point of view. The misinterpretation presumably was caused by reading as 'responses of an individual axon' the words "responses of individual axons". In so far as the doctrine of specific nerve energies is concerned, the point we wished to make, and would have made in as many words had there been space in an abstract, was that since it is possible to distinguish in the fibers of a nerve a very wide and apparently continuous range in the characters of their responses the possibility exists of a transmission of impulses along paths in the central nervous system adapted to impulses of specific configurations. This is an elaboration, based on more definite information, of a point of view previously presented by Erlanger, Gasser and Bishop[3] and since by Adrian.[4]

The differences between their and our *results,* to which they refer more briefly, will be considered in a paper to be published elsewhere.

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[3] Erlanger, J., Gasser, H. S., and Bishop, G. H., *Am. J. Physiol.*, 1924, **70**, 665.

[4] Adrian, E. D., "The Mechanism of Nervous Action," 1932, 56 *et seq.*

7026 P

Amino Acid Metabolism. Fate of dl-Methionine in the Phlorhizinized Dog.

HARRY M. VARS. (Introduced by L. B. Mendel.)

From the Laboratory of Physiological Chemistry, Yale University, and the Biological Laboratory, Princeton University.

The manifest interest in the metabolism of methionine makes it desirable to record the fate of this amino acid in phlorhizin diabetes. The generalizations to be derived from the fate of other amino acids (Lusk[1]) leads one to suspect that methionine can act as a glucose former. The data to be presented indicate that dl-methionine can be metabolized to yield glucose in the phlorhizinized dog.

dl-Methionine was synthesized by the method of Windus and Marvel.[*][2] Phlorhizin was administered by the Coolen[3] method. Urine was collected by catheterization. Nitrogen was determined by the usual Kjeldahl method, glucose by the Benedict method as described by Quick,[4] and sulfur and sulfates by the methods of Folin.[5] The amino acid was administered as the sodium salt, being injected subcutaneously in a volume of 140 cc. The data are summarized in Table I.

The sulfur is excreted slowly. Dakin[6] found similarly that the

TABLE I
Glucose Formation from Injected dl-Methionine in Phlorhizin Diabetes.

Period	hr.	Wt. Kilo	N Gm.	Glucose Gm.	D/N	Total S Gm.	Inorg. SO_4-S Gm.	Total SO_4-S Gm.	Extra Glucose Theory 3-Carbon Gm.	Extra Glucose Found Gm.
I*	24	7.7	6.00	20.56	3.42	.433	.285	.298		
II	24	7.5	7.09	23.00	3.25	.476	.292	.315		
III	12	7.1	4.36	15.16	3.48	.270	.158	.167		
IV†	12	7.3	4.68	17.56	3.75	.788	.306	.334	7.50	5.76
V	24	6.7	8.00	27.20	3.40	1.637	.718	.742		
VI	12	6.5	3.09	10.54	3.41	.429	.232	.250		

* Dog starved 3 days—receiving phlorhizin (1 gm.) for 1 day prior to period 1. Phlorhizin injected daily thereafter.

† 12.5 gm. dl-methionine injected subcutaneously (1.17 gm. N. 2.69 gm. S).

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[1] Lusk, G., The Science of Nutrition, W. B. Saunders Co., 4th Ed., 1928.

[*] The amino acid was prepared in collaboration with Dr. R. W. Jackson, following supplementary directions received directly from Dr. Marvel.

[2] Windus, W., and Marvel, C. S., *J. Am. Chem. Soc.*, 1930, **52**, 2575.

[3] Coolen, F., *Arch. f. Pharm.*, 1895, **1**, 267.

[4] Quick, A. J., *Ind. Eng. Chem.*, 1925, **17**, 729.

[5] Hawk, P. B., and Bergeim, O., Practical Physiological Chemistry, P. Blakiston Son & Co., 9th Ed., 1926, 770.

[6] Dakin, H. D., *J. Biol. Chem.*, 1913, **14**, 321.

sulfur excretion after cysteine injection was relatively slow, recovering about 50% of the sulfur of the amino acid in the urine during 36 hours following the injection. In contrast to the results with cysteine the major portion of the methionine sulfur was excreted in the neutral sulfur fraction. The post-injection urines gave a positive reaction with Grote's[7] test for C-S-S-C compounds. The Sullivan test for cystine, as described by Brand, Harris and Biloon[8] gave questionable results. The presence of acetone bodies in the urine prevented the interpretation of the usual sodium cyanide-sodium nitroprusside test for S-S compounds.

7027 C

On the Mechanism of Sodium Depletion in Addison's Disease.

ROBERT F. LOEB, DANA W. ATCHLEY, ETHEL B. GUTMAN AND RUTH JILLSON.

From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York City.

The authors have made the following observations in relation to the development of acute adrenal insufficiency in Addison's disease: 1. There is a marked decrease in the concentration of sodium in the blood serum.[1] 2. The withdrawal of salt from the diet of a patient suffering from Addison's disease promptly induces symptoms of severe insufficiency.[2] 3. The administration of NaCl in large amounts brings about striking clinical improvement which may be correlated with a return of the Na content of the blood to a normal level.[1, 2] These findings have recently been confirmed by Harrop and his coworkers.[3, 4] 4. Sodium is lost from the body of adrenalectomized dogs as a result of augmented renal excretion of this element.[5] This observation has also been substantiated by Harrop.[6]

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[7] Grote, I. W., *J. Biol. Chem.*, 1931, **93**, 25.

[8] Brand, E., Harris, M. M., and Biloon, S., *J. Biol. Chem.*, 1930, **86**, 315.

[1] Loeb, R. F., *Science*, 1932, **76**, 420.

[2] Loeb, R. F., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 808.

[3] Harrop, G. A., Weinstein, A., Soffer, L. J., Trescher, J. H., *J. Am. Med. Assn.*, 1933, **100**, 1850.

[4] Harrop, G. A., *J. Am. Med. Assn.*, 1933, **101**, 388.

[5] Loeb, R. F., Atchley, D. W., Benedict, E. M., Leland, J., *J. Exp. Med.*, 1933, **57**, 775.

[6] Harrop, G. A., Soffer, L. J., Ellsworth, R., and Trescher, J. H., *J. Exp. Med.*, 1933, **58**, 17.

The writers have suggested that the adrenal glands play an important rôle in the regulation of sodium metabolism, and that the sodium depletion occurring in adrenal insufficiency is dependent therefore upon a disturbance of this function. This idea gains support from the fact that in adrenal insufficiency there is a loss of sodium without change in potassium excretion, whereas in other clinical states associated with base loss, such as ketogenic acidosis due to starvation[7] or diabetes,[8] NH_4Cl acidosis,[9] and severe diabetic glycosuria,[8] sodium depletion is accompanied by a parallel loss of potassium. The foregoing facts suggest that the kidney is the locus of action of the sodium regulatory mechanism of the adrenal gland.

It remained to be shown, however, that sodium is not lost from the body in adrenal insufficiency as the result of an acidosis due to the formation and excretion of some undetermined organic acid. For this reason we have studied the excretion of ammonia and titratable acid in a patient suffering from Addison's disease, in whom acute adrenal insufficiency was induced by salt withdrawal and in whom "compensation" was reestablished by salt administration. Since it has been shown[8] in diabetic ketosis that the NH_3 excretion increases 10 fold, reaching more than 225 m. eq. in 24 hours and the titratable acid of the urine is correspondingly augmented, a complete balance study was not considered essential for the observations here presented, particularly as the decrease in sodium concentration in the blood in acute adrenal insufficiency is even greater than that found in severe acidosis.

The patient, E. F., was a white woman of 38 whose past history was irrelevant except for tuberculosis of the hip and pleurisy with effusion. For 2 years before admission she had irregular abdominal pains and occasional vomiting spells. For the past year there had been rapidly increasing brown pigmentation of the whole body, and for 6 months she had had dyspnoea on exertion and definite but not marked weakness. Physical examination was entirely negative except for extensive generalized brown pigmentation. There were, however, no patches in the buccal mucous membranes. Her blood pressure varied between 112/80 and 76/50 during her stay in the hospital.

The patient was given a regular diet and was allowed to eat salt as she desired, for eighteen days, during which time the patient

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[7] Gamble, J. L., Ross, G. S., Tisdall, F. F., *J. Biol. Chem.*, 1923, **57**, 633.

[8] Atchley, D. W., Loeb, R. F., Richards, D. W., Jr., Benedict, E. M., and Driscoll, M. E., *J. Clin. Invest.*, 1932, **12**, 297.

[9] Folling, A., *Acta. Med. Skandinav.*, 1929, **71**, 221.

TABLE I

Date	Urine Analysis				Blood Analysis							Remarks
	Vol. cc.	Cl m. eq.	Titratable acid cc. $\frac{N}{10}$ acid	NH_3 m. eq.	Na m. eq. per l	K m. eq. per l	Cl m. eq. per l	HCO_3 m. eq. per l	Protein %	Non Protein N mg. per 100 cc.	Glucose gm. per l	
Aug. 18					135.3	4.6	100	25.3				Admission blood. Patient. quite well.
" 21-22	818	82.6	121	15.2								Regular diet until 9/5.
" 22-23	1667	124.0	172	15.5								
" 23-24	1790	136.9	221	18.1								
" 24-25	1240	112.5	174	13.9								
" 25-26	885	84.1	151	13.6								
" 26-27	1660	116.0	205	20.5								Catamenia
" 27-28	1615	129.5	183	23.2								"
" 28-29	1355	110.5	216	23.8								"
" 29-30	1210	92.6	219	18.7								"
" 30-31	1510	98.8	137	11.6								
Aug. 31-Sept. 1	2124	166.0	142	15.4								
Sept. 1- 2	1310	153.0	189	13.7								
" 2- 3	1610	147.5	100	10.3								
" 3- 4	2008	183.0	171	9.6								
" 4- 5	2310	159.0	213	11.0								Weaker than on admission
" 5- 6	1477	106.8	157	11.9	131.0	5.1	97.3	26.2	7.5	30.3	0.91	Salt poor diet started 9/5.
" 6- 7	965	55.0	129	13.3								Marked prostration. Unable to get out of bed.
" 7- 8	1847	178.0	190	11.2	123.4	5.6	88.9	25.9	7.8	26.7		Picture of severe adrenal insufficiency before salt administration, which started after blood was taken on 9/7.
" 8 -9	2034	352.0	214	14.5								
" 9-10	2160	290.5	221	20.5								
" 10-11	1710	350.1	241	19.6								
" 11-12	3605	376.0	316	23.7								
" 12-13	3290	320.0	285	26.2								
" 13-14	2844	417.0	270	26.7								
" 14-15	1760	300.7	214	20.7	138.1	4.9	105.0	25.7	5.6	22.7	0.81	"Feels perfectly well." Taking 10 gm. NaCl daily plus diet.

was up and about the ward, though she complained of nausea, occasional abdominal pain and slowly increasing weakness. She was then given a regular but salt-poor diet, containing less than 2.0 gm. of NaCl a day. Within 24 hours weakness and nausea increased, she felt dizzy, and had to remain in bed. The next day prostration was more marked, nausea was extreme and she vomited a total of 210 cc. in 48 hours. The patient's state of prostration did not justify continuation of the salt-poor regime, and in the next 24 hours she received a total of 20 gm. of NaCl by mouth and by rectum, in addition to the salt of her diet. Within 2 days she was up and about once more and was discharged 5 days later essentially symptom-free, taking 10 gm. of salt daily, in addition to that in her food.

The results are shown in the accompanying table. During the first 18 days of observation, while the patient ate salt as she desired, the sodium of the blood decreased from 135.3 m. eq. per liter to 131 m. eq. Coincident with this slow, steady loss of sodium, the NH_3 excretion and the titratable acidity of the urine were maintained at a normal level for the usual ward diet. During the next 48 hours when salt was withheld from the diet, the blood sodium fell abruptly to 123.4 m. eq. per liter, without increase in the excretion of ammonia or titratable acid. As sodium administration was forced (10 to 20 gm. of NaCl daily, in addition to salt of diet) the ammonia excretion increased slightly, but did not approach a level that would indicate significant acidosis. Furthermore, in this patient, the blood bicarbonate concentration was normal throughout the entire period of observation, in the presence of the marked fluctuations in the sodium level.

From this brief study it is apparent that the loss of sodium from the body in Addison's disease is not dependent upon the excretion of unusual quantities of acid as determined by the excretion of NH_3 and titratable acid, and hence it seems probable that we are dealing with a primary disturbance of the sodium regulatory mechanism, as the writers have previously suggested.[5] From the data now available, it may be inferred that the essential feature of this disturbance is an increase in the rate of sodium excretion by the kidney.

In this patient, as previously reported,[2] the clinical condition parallels the level of sodium in the blood, and the state of well-being is largely dependent upon the amount of sodium ingested, as might be predicted from the foregoing discussion.

7028 C

Ceryl Alcohol from the Grass, Agrostis.

CHARLES E. BILLS AND GODFREY E. STEEL.

From the Research Laboratory, Mead Johnson and Company, Evansville, Indiana.

Bondzynski and Humnicki[1] isolated from horse dung an unsaponifiable substance which they named hippocoprosterol. It was assumed to be a reduced sterol, analogous to coprosterol, but representing a greater degree of reduction. It was later obtained, impure, by Wilenko.[2] Dorée and Gardner[3] observed that hippocoprosterol from the horse, cow, sheep, and rabbit is not a product of sterol metabolism, but is merely an unaltered constituent of the grass ingested by these herbivora. Considering its origin, they renamed it chortosterol.

Collison and MacLean[4] observed recently that the alcoholic constituent of spinach leaves is identical with the ceryl alcohol of Chinese insect wax. Similarity in physical properties and percentage composition suggested to us that all these products are essentially one and the same substance. Experiments were begun with the grass known as bent, but they were discontinued after we saw the comprehensive report by Pollard, Chibnall and Piper.[5] These workers indicated that hippocoprosterol is ceryl alcohol, the latter being defined as a fatty alcohol mixture having a mean carbon content of about C_{27} and a melting point of about 80°. In cocksfoot and perennial ryegrass they found the ceryl alcohol to consist predominantly of *n*-hexacosanol.

Our limited experiments with a third species of grass confirm this work. The bent, *Agrostis* (Sp.), was obtained as clippings from a putting green almost free of other vegetation. Air-dried, it was extracted with hexane, and the crude wax was boiled with alcoholic KOH. The solution was treated with alcoholic $CaCl_2$, filtered hot, and cooled to 20°. The crude ceryl alcohol was treated with charcoal in hexane, and again in benzene. The yield, after losses, was about 1% of the dry grass.

This ceryl alcohol melted at 80.3°. Ceryl alcohol from Chinese insect wax was similarly prepared, except for no charcoal treatment. It also melted at 80.3°. The 2 in admixture gave no depression of melting point. These melting points disregard the persistence of a "ghost" which, in the grass alcohol lasted for nearly 3°, and in the insect wax alcohol for about 2° above the figures given. The alcohol from grass solidified at a slightly lower temperature than that from insect wax, and it was also slightly less soluble in ether. Both specimens showed only general absorption in the ultraviolet region, that from grass being somewhat the more opaque. In other respects the 2 were indistinguishable. It appears that the ceryl alcohol from *Agrostis* is essentially the same substance, perhaps with a slightly higher mean carbon content, as that from the 2 grasses first investigated.

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[1] Bondzynski, S., and Humnicki, V., *Z. physiol. Chem.*, 1896, **22**, 396.

[2] Wilenko, M. G. G., *Bull. internat. Acad. Sci. Cracovie*, 1906, 20.

[3] Dorée, C., and Gardner, J. A., *Proc. Roy. Soc. London, ser. B*, 1908, **80**, 212.

[4] Collison, D. L., and MacLean, I. S., *Biochem. J.*, 1931, **25**, 606.

[5] Pollard, A., Chibnall, A. C., and Piper, S. H., *Biochem. J.*, 1931, **25**, 2111.

7029 C

Inhibition of Hypoglycemic Perspiration by Spinal Anesthesia.

HARRY KOSTER AND ARTHUR I. MIRSKY.* (Introduced by W. S. Collens.)

From the Research Laboratories of the Crown Heights Hospital.

When the blood sugar falls to a critical level, many manifestations of sympathetic excitation present themselves in the so called hypoglycemic syndrome. The investigations of Cannon, McIver and Bliss[1] with the denervated heart, of Abe[2] with the denervated iris, of Houssay, Lewis and Molinelli[3] with their cross-circulation preparations, and others,[4] indicate that insulin hypoglycemia is associated with a discharge of epinephrine from the adrenal medulla. Inhibition of the sympathetic adrenal mechanism by any means (sympathectomy, splanchenectomy adrenal medulla ablation, ergotamine poisoning) prolongs the hypoglycemia. This indicates that epinephrine plays an important rôle in the regulation of and in compensation for any abnormal diminution of the blood sugar.

In man, we have no direct evidence that the phenomena observed during hypoglycemia are the result of excitation of the sympathetico-adrenal mechanism; our evidence is only inferred from the animal experimentation. During the course of studies on hypoglycemia experimentally produced in man, we observed that one of the earliest and prominent symptoms of a marked fall in the blood sugar, was profuse perspiration. The sweat glands receive nerve fibres which are entirely sympathetic in origin (Langley[5]). It is therefore not surprising that a mechanism apparently which excites the sympathetico-adrenal mechanism should produce profuse perspiration. That this is a "cause and effect" relationship can only be inferred. We could find no direct proof in the literature. To test the hypothesis we attempted to inhibit the sympathetic nervous system, employing spinal anesthesia for the purpose.

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* Richard Morton Koster Fellow.

[1] Cannon, McIver and Bliss, *Am. J. Physical,* 1924, **69**, 46.

[2] Abe, *Arch. Exp. Path. Pharm.,* 1924, **103**, 73.

[3] Houssay, Lewis and Molinelli, *Compt. rend. Soc. Biol.,* 1924, **91**, 1011.

[4] MacLeod, Carbohydrate Metabolism and Insulin.

Spinal anesthesia is associated with many manifestations of sympathetic inhibition, *viz.*, vasodilatation, intestinal contractions, hypotension, decreased heat rate, etc. The anesthesia *per se,* does not induce perspiration. We therefore employed the following procedure: 0.1 unit of insulin per kilo of body weight was administered to subject and the extent of perspiration noted. The sweating usually began within a half hour. On the following day the patient was given a spinal anesthesia through the intratracheal administration of neocaine. As soon as anesthesia was complete, insulin was administered and the extent of sweating again noted. In some cases, blood samples were taken in oxalate at intervals throughout the experiment for blood sugar determinations. In every instance where the skin anesthesia was below the level of the fifth dorsal segment ("low anesthesia") perspiration occurred only above the area of anesthesia. The anesthetic area always increased in temperature but showed no detectable perspiration even in the presence of profuse large beads of sweat in the non-anesthetized area. In some cases, the hypoglycemia occurred at the same time as the level of skin anesthesia diminshed. In these instances, the area of perspiration gradually increased.

These observations indicated that a paralysis of the sympathetic nervous system, *per se,* may be responsible for the lack of perspiration in the anesthetized areas, and that an increase in circulating epinephrine was not a direct factor in the peripheral manifestations of the hypoglycemic syndrome.

To further elucidate this question, a normal male was given 4 units of insulin intravenously and in 18 minutes he began to perspire. Beads of perspiration became visible over the whole skin surface. On the following day 300 mg. of neocaine was administered intraspinally and a "high" anesthesia was obtained. The anesthetic area extended to just above the fifth dorsal segment. Insulin was then administered intravenously. The subject was watched carefully for 2 hours and though his blood sugar fell to 43 mg. %, no visible perspiration was noted anywhere. In this case the site of origin of all the sympathetic fibres was affected by the spinal anesthetic. However, the experiment does not permit the presumption that adrenalin secretion is not responsible for the hypoglycemic perspiration. It is probable that the splanchnic nerves to the adrenals were anesthetized and that therefore these glands were denervated functionally. As a consequence, the hypoglycemia could not stimulate adrenalin secretion through these nerves. If the latter is true it is still impossible to explain on this basis the absence of perspiration in the anesthetized areas during a "low spinal anesthesia."

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[5] Langley, *J. Physical,* 1891, **21**, 347.

Adrenalin stimulates "nerve-endings", hence any increase of this hormone in the circulating blood should be associated with peripheral manifestations of its action even if the nerve supplying the part is removed. To test this concept we studied 7 patients who had been subjected to lumbar sympathectomies for various diseases. The following case is illustrative of the general results. M. G., a male, aged 28, suffering from early thromboangiitis obliterans was given an intravenous injection of 0.1 units insulin per kilo of body weight. In 23 minutes he began to perspire profusely, both feet showing a similar degree of moisture. He was subsequently subjected to a unilateral lumbar sympathectomy. Two weeks post operation, he was again subjected to the above procedure but no perspiration was noted in either leg. Subsequent studies revealed that 2 or 3 times the preoperative dose of insulin was necessary to produce the hypoglycemic syndrome. The degree of perspiration was similar on both sides in the majority of instances but several cases showed more sweating on the normal side.

It is therefore apparent that insulin hypoglycemia can induce a secretion of the sweat glands even in the complete absence of the sympathetic nerve to the glands.

These studies are reported because they indicate a definite rôle of the sympathetic nervous system in the insulin hypoglycemic syndrome.

Numerous explanations of the above observations may be made. However, two possible mechanisms merit further discussion. The low and high anesthesia studies suggest that hypoglycemia induces a stimulation of the sympathetic nervous system caudal to the spinal cord, probably in the hypothalamus; that the stimulation is transmitted via the splanchnic nerves to the adrenals and that the consequent increase in adrenalin secretion produces the observed peripheral effects. This hypothesis does not explain all the observations. The other possible mechanism suggested, is a primary, direct stimulation of the adrenals by the low blood sugar, with a consequent increase in circulating adrenalin which in turn stimulates the sympathetic nervous system centers in the midbrain. Anesthesia of the sympathetic nerves prevents the transmission of these impulses to the sweat glands and perspiration does not occur. When the amount of circulating adrenalin is markedly increased then the effects are produced by peripheral stimulation of the nerve endings.

7030 C

Direction of Amoeboid Movement of Leucocytes on a Glass Surface in an Electric Field.

WILLIAM B. WARTMAN AND MORTON MC CUTCHEON.

From the Department of Pathology, University of Pennsylvania School of Medicine.

It is well known that in inflammatory areas the hydrogen ion concentration is frequently increased.[1] According to Abramson,[2, 3] the chemical changes in tissues incidental to injury would give rise to differences in electric potential sufficient to account for emigration of leucocytes. Migration in such an electric field might be due in part to electrophoresis, in part to amoeboid movement of which the direction was determined by the electric field.[4]

The present report is concerned only with the latter manner of progression, amoeboid movement in an electric field, a phenomenon that has been termed, somewhat loosely, galvanotaxis. It seemed of some interest to those of us who are concerned with the general pathology of inflammation and especially with the mechanism of chemotropism to learn whether the amoeboid motion of leucocytes can in fact be directed by an electric current, and, if so, toward which pole the cells move.

Only two papers on galvanotaxis of leucocytes have been found in the literature. Mendelssohn[5] reported that the cells moved toward the cathode, Feringa,[6] that they went toward the anode.* The latter of these brief reports does not make it clear whether the author was actually observing the effect of galvanotaxis or electrophoresis. Yet the distinction is important for the present discussion. In electrophoresis the cell is passively transported by electric forces through the liquid or jelly[3] in which it is suspended. In galvanotaxis the cell is actively crawling on a solid support (a glass slide); the electric current merely determines the direction in which it crawls.

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[1] Schade, H., Neukirch, P., and Halpert, A., *Z. Exp. Med.*, 1921, **24**, 11. For brief review of literature see Loos, H. O., *Z. Exp. Med.*, 1931, **75**, 463.

[2] Abramson, H. A., *J. Exp. Med.*, 1927, **46**, 987.

[3] Abramson, H. A., *J. Gen. Physiol.*, 1928, **11**, 743.

[4] Abramson, H. A., in Alexander's Colloid Chemistry, New York, 1928, **2**, 701.

[5] Mendelssohn, M., *Comptes rend. Acad. des Sciences*, 1916, **162**, 52.

Another distinction may be drawn. In electrophoresis the mobility or bodily movement is proportional to the applied electric potential, whereas in galvanotaxis, no effect is evident for small potentials, the effect being easily observed only when the current density exceeds a minimal value.

The apparatus in the present experiments was similar to the one used by Hahnert[7] in studying amoebae. At each end of an ordinary glass slide is placed a paraffin cup containing aqueous copper sulphate solution, into which dip copper electrodes. Bridges of agar jelly (agar 2%, NaCl 0.8%) conduct the current from the paraffin cups to very thin, moist strips of filter paper. These strips pass under opposite edges of a coverslip. A drop of cell suspension (exudative rabbit polymorphonuclear leucocytes resuspended in serum) is placed between coverslip and slide and the preparation is sealed with vaseline. It is placed on the stage of a microscope in a warm box and observed with the oil immersion lens. In these experiments an E.M.F. of 45 volts was used. The field strength was not ascertained.

When a new preparation is desired, the electrode cups and agar bridges are moved onto a fresh slide, fresh strips of filter paper are put in position and a drop of cell suspension mounted between slide and coverslip. Such a preparation can be made in less than 5 minutes.

The cells are first observed with the current off. After a few minutes amoeboid motion begins and the cells wander over the slide at random. The current is then turned on, but for about 2 minutes the crawling cells continue to move at random, showing no response to the current, though cataphoresis of suspended particles begins at once. After about 2 minutes the crawling cells begin to move toward the negative pole or cathode. Observation with the oil immersion lens reveals no change in the character of amoeboid movement, except in its direction. The cells are not floating, but are attached to the glass slide. Protoplasmic streaming, as revealed by movements of the cell granules, is normal and does not suggest that the cell is being dragged over the glass by the electro-osmotic stream.

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[6] Feringa, K. J., *Arch. néer. physiol.*, 1925-6, **10**, 406.

* Amoebae generally move toward the cathode.[7]

[7] Hahnert, W. F., *Physiol. Zool.*, 1932. **5**, 491.

When the current is reversed, the cell continues to move in the same direction as before, that direction being now toward the anode. However, after a minute the cell gradually turns around until, after about 2 minutes, it has reversed its direction and is moving once more toward the cathode. This result was always obtained as long as the cells remained in good condition.

The accompanying camera lucida drawing shows the changes in direction of a representative leucocyte.

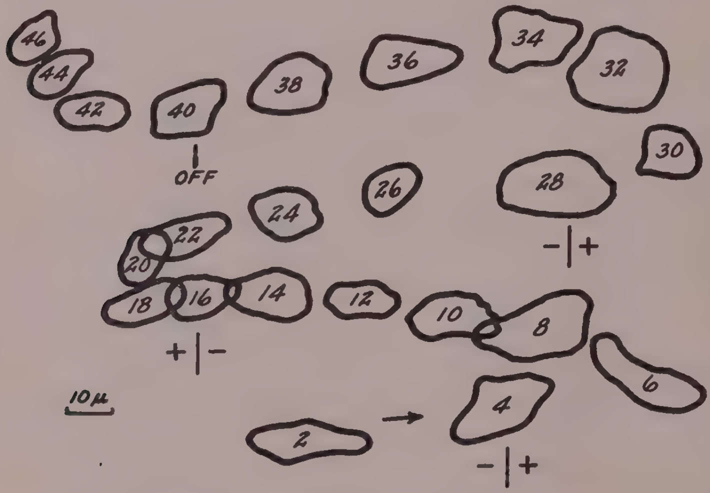

Fig. 1.

Camera lucida drawings of a single leucocyte at minute intervals. When the leucocyte was at position 4 the current was turned on, with the negative pole to the left. The cell gradually turned around and went to the negative pole. This response was repeated where the current was reversed with the cell at position 16 and again at position 28. At position 40, the current was turned off.

It is concluded that under these conditions exudative polymorphonuclear leucocytes of the rabbit, placed in an electric field, progress by amoeboid motion toward the cathode. These experiments do not allow us to decide whether the cells are responding to electric polarization (galvanotaxis), to stimulation by the electro-osmotic flow of water, or to other factors that might determine the direction of amoeboid movement.

Evidently cells in an electric field may show either of 2 types of locomotion: first, electrophoretic movement, which is easily observable at low field strength, forcing the leucocyte toward the anode[2]; second, amoeboid galvanotactic movement in higher field strengths, forcing the leucocyte in the opposite direction toward the cathode.

Whether these 2 factors are of importance in determining the direction of locomotion of leucocytes in an inflammatory area remains to be determined.

7031 C

A Thermoelectric Blood Flow Recorder in the Form of a Needle.

F. A. GIBBS. (Introduced by D. W. Bronk.)

From the Eldridge Reeves Johnson Foundation, University of Pennsylvania School of Medicine.

The needle flow recorder described here was primarily designed to measure rate of blood flow through the internal jugular vein of man. Preliminary work on animals has shown that the device possesses certain characteristics that would seem to promise it a sphere of usefulness in a variety of problems where blood flow is an important variable. The advantages of the device are as follows: Its use entails only a minimal trauma. It can be applied to the study of flow through relatively inaccessible structures. The method makes it possible to measure qualitatively the changes in blood flow through a vascular tissue without isolating or cannulizing the afferent or efferent vessels, and thus enables one to study the blood flow through a tissue or organ, the blood supply of which is not capable of being completely isolated. It also makes possible relative determinations of the blood flow through various parts of the same organ or region. This has not been possible by any method previously described. The thermoelectric methods for measurement of volume flow of blood previously reported require cannulization of a vessel and the passing of heparinized blood through a water jacket, the temperature of which is thermoelectrically determined (Bronk and Gesell[1]); or the isolation of a vessel, the heating of the blood therein by means of a high frequency current, and the determination of the temperature gradient along the vessel (Rein[2]); or the insertion of an organ or region into a calorimeter.

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[1] Gesell and Bronk, *Am. J. Physiol.*, 1926, **79**, 61.

The theory upon which the instrument operates is extremely simple. The cooling power of a stream depends, among other things, upon its velocity. If a needle, supplied with a constant amount of heat, is thrust into a vessel or into a tissue through which blood is flowing, the temperature of the needle will vary inversely with the flow of blood. That is, an increased flow of blood will cool the needle, and a decreased flow will allow the needle to heat up. By measuring the temperature of the needle it is possible to obtain an index of the blood flow. The tip of the needle can be supplied with a constant amount of heat by incorporating in it a length of high resistance wire through which a constant electric current is allowed to pass. The temperature of the tip of the needle can be conveniently measured by means of thermojunctions in series with a galvanometer, one junction being mounted in the tip of the needle.

The construction of the needle and the circuits used are shown in Fig. 1. Dimensions of the wires and spacing of the connections varied with the size of the needle and with the particular situation in which the needle was to be employed. One copper lead was usually

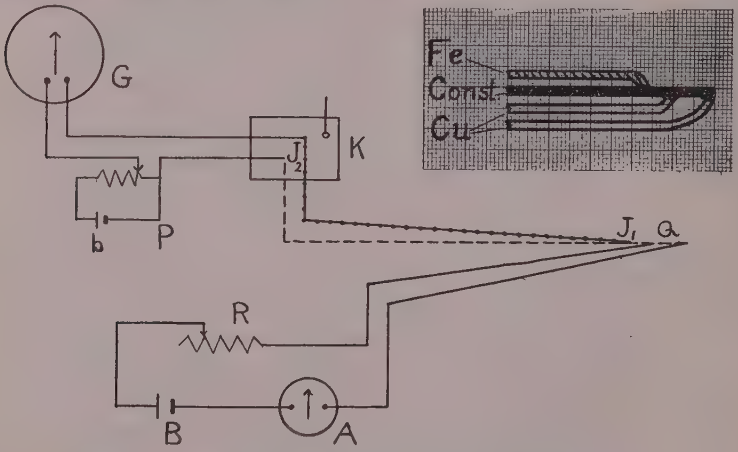

FIG. 1. Wiring diagram of needle blood flow recorder.

Solid line, copper wire; dotted line, iron wire; broken line, constantan wire. J_1 and J_2, thermojunctions; K, constant temperature box; P, potentiometer with b, source of known E. M. F.; G, galvanometer; Q, length of constantan used as heating element; A, milliammeter; B, battery; R, variable resistance. Insert shows large scale drawing of needle tip.

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[2] Rein, *Z. f. Biol.*, 1928, **87**, 394.

connected to the constantan wire at the very tip of the needle (see large scale insert in Fig. 1); the second copper lead was soldered to the constantan about 3 mm. behind the first; and the iron wire was soldered to the constantan a millimeter or less behind the second copper lead. Thirty-six gauge copper, iron, and constantan wire were used in making large needles. Wire .02 mm. in diameter was used in making the finest needles. Electrical insulation and heat insulation were obtained by covering the wires with bakelite varnish. The heating current was supplied by a 6 volt storage battery in series with a suitable rheostat and ammeter. A potentiometer, capable of measuring tenths of a millivolt, was employed in conjunction with a sensitive galvanometer to measure the E. M. F. in the thermojunction circuit. Running records of the E. M. F. in the thermojunction circuit were made by recording on a smoked drum the turning of the potentiometer dial (which was manually

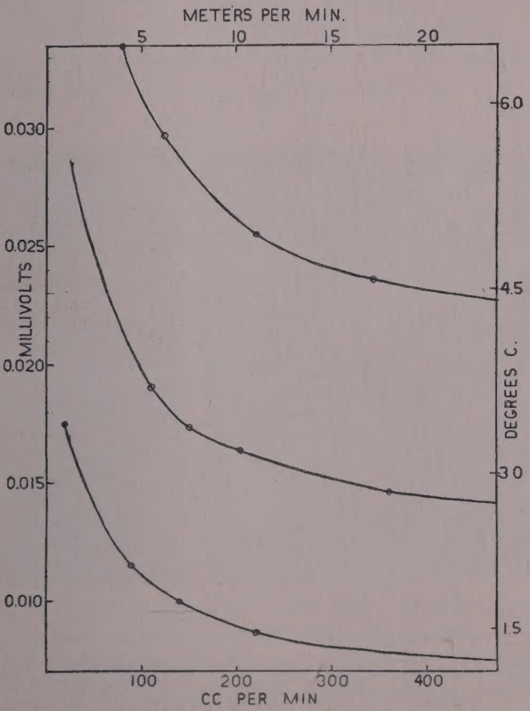

Fig. 2. *In vitro* calibration of needle flow recorder.

The curves give the E. M. F. and the corresponding difference of the temperature of the needle tip compared to that of the unheated blood for different rates of flow (lower scale minute flow, upper scale velocity). The 3 curves were obtained for 3 different strengths of the heating current.

operated) or by partially balancing the thermojunction current with the potentiometer and following the reflected light beam from the galvanometer mirror with a pointer mounted on a carriage under the galvanometer scale, the movement of the pointer being communicated to a writing arm recording on a smoked drum.

When the needle tip was placed in a stream of defibrinated ox blood flowing through a glass tube, the temperature of the needle varied with the flow and with the amount of heat supplied in the manner shown in Fig. 2.

Fig. 3 shows the change that occurs in blood flow through the kidney when adrenalin is injected intravenously. Records of changes in blood flow due to electrical stimulation of vasomotor nerves are shown in Fig. 4.

Experience has indicated that in anesthetized animals changes in body temperature do not introduce a serious source of error. By making alternate readings of the temperature of the needle tip, with the heating current off and with the heating current on, a change in body temperature can be noted and corrected for. In unanesthetized animals violent muscular exertion and emotional excitement produce sudden changes in body temperature. These can be automatically corrected for, if instead of placing the cold junction in a constant temperature box, it is placed in the tissue with the needle. The danger that changes in flow in distant areas may be misinterpreted as occurring at the needle tip can be minimized by using a small amount of heat, and by reducing the heat conductivity of the needle. Satisfactory sensitivity can be obtained without raising the temperature of the needle more than 2°C. above body temperature.

Whenever it is possible to maintain constant the cross section area of the stream being measured, the instrument can be calibrated (preferably *in situ*) so as to yield quantitative data. An example of such a situation is the bony canal that forms the lateral sinus in the dog. When the instrument is calibrated in an ordinary blood vessel, it should be realized that the changing pressures alter the cross section area of the vessel and make an accurate calibration impossible. Rough quantitative data can be obtained if the needle is calibrated in tissue. For example a record may be made of the E. M. F. in the thermojunction circuit with the needle flow recorder in the cortex of a cat's kidney. Various experimental procedures may be carried out and the resulting changes in E. M. F. recorded. At the end of the experiment which may last 2 or 3 hours, during all of which time it is possible to keep a running record of the E. M. F., a stromuhr can be inserted into the renal vein. By plot-

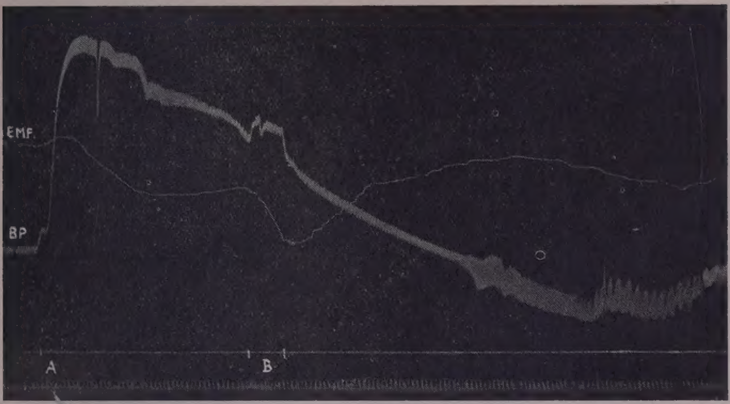

Fig. 3. Change in Blood Flow Through the Kidney Produced by Intravenous Injection of Adrenalin.

Needle flow recorder in cortex of the kidney of a cat anesthetized with sodium amytal. At A 1 cc. of 1:10,000 solution of adrenalin was injected intravenously. At B loose ligature around abdominal aorta above renal artery was pulled upon. Blood pressure tracing taken with cannula in common carotid artery. Time in 5 secs. Rate of blood flow past tip of needle indicated by E. M. F. in galvanometer circuit. Movement of the writing arm toward the base line shows increase of E. M. F. and a decrease in the rate of flow.

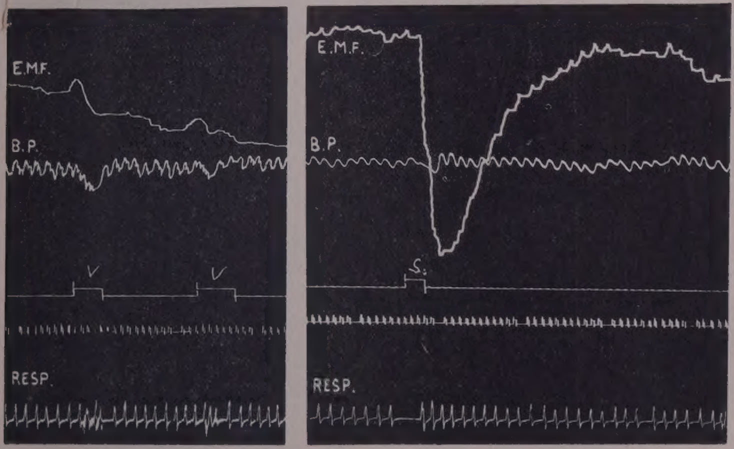

Fig. 4. Change in Blood Flow Through the Temporal Muscle Produced by Stimulation of Vagus and Sympathetic Nerves.

Needle blood flow recorder in muscle of a cat. Sodium amytal anesthesia. At V central end of homolateral vagus nerve stimulated with weak induction shock. At S central end of homolateral cervical sympathetic trunk similarly stimulated. Blood pressure cannula in femoral artery. Records as in Fig. 3.

ting E. M. F. against cc. of blood per min. as measured with the stromuhr, a curve is obtained which makes it possible to interpret the previously obtained record of E. M. F. in terms of flow through the whole kidney. Such a curve is shown in Fig. 3. The most serious source of error in such a procedure lies in the possibility that the relationship between the total flow through the kidney and the fraction of that flow which affects the temperature of the needle may have altered in the course of the experiment.

Though it is thus possible in certain situations, and by taking proper precautions, to obtain data with this instrument that are susceptible of quantitative interpretation, it is most advantageously used when employed as an essentially qualitative instrument. Such an instrument is not without present value in medicine and physiology. There remain important problems relating to blood flow that do not require for their solution knowledge of the actual volume flow per minute, but only require information as to whether the flow is slightly increased, greatly increased, continues unchanged, or is diminished. It was for such problems that this instrument was primarily designed.

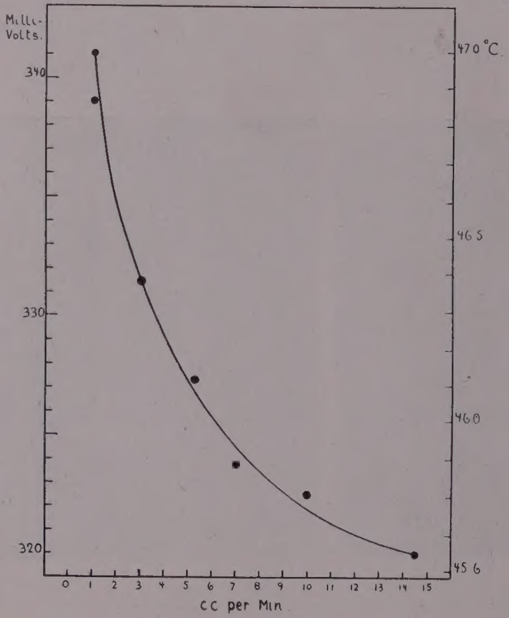

FIG. 5. *In vivo* calibration of needle blood flow recorder in cortex of cat's kidney against stromuhr.

Inserted into renal vein. Amytal anesthesia. Flow through kidney decreased by pulling on loose ligature around renal artery. It will be observed that an excessive amount of heat was supplied to the needle tip. This was an early experiment; later experience showed that satisfactory sensitivity can be obtained without raising the temperature of the needle tip more than 2°C. above body temperature.